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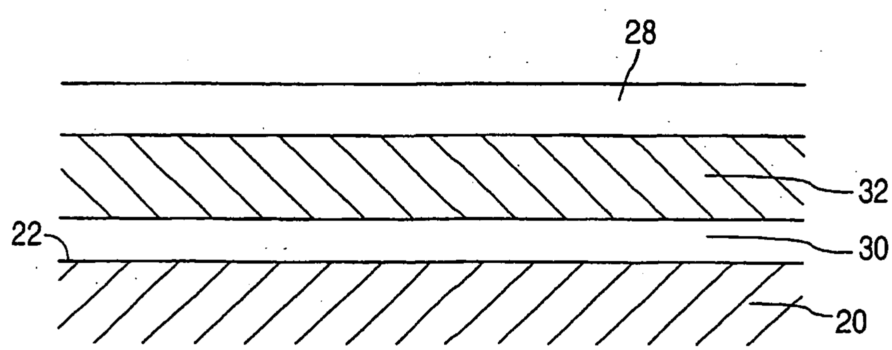
(54) Title: A RATE-REDUCING MEMBRANE FOR RELEASE OF AN AGENT



(57) Abstract: A membrane that reduces the rate at which a therapeutic substance is released from an implantable medical device, such as a stent, is disclosed.

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A RATE-REDUCING MEMBRANE FOR RELEASE OF AN AGENT

BACKGROUND OF THE INVENTION

Field of the Invention

The invention relates to a coating disposed on an implantable device, one example of which is a stent, for reducing the release rate of an agent carried by the device.

Description of the Background

Percutaneous transluminal coronary angioplasty (PTCA) is a procedure for treating heart disease. A catheter assembly having a balloon portion is introduced percutaneously into the cardiovascular system of a patient via the brachial or femoral artery. The catheter assembly is advanced through the coronary vasculature until the balloon portion is positioned across the occlusive lesion. Once in position across the lesion, the balloon is inflated to a predetermined size to remodel the vessel wall. The balloon is then deflated to a smaller profile to allow the catheter to be withdrawn from the patient's vasculature.

A problem associated with the above procedure includes formation of intimal flaps or torn arterial linings, which can collapse and occlude the conduit after the balloon is deflated. Vasospasms and recoil of the vessel wall also threaten vessel closure. Moreover, thrombosis and restenosis of the artery may develop over several months after the procedure, which may necessitate another angioplasty

procedure or a surgical by-pass operation. To reduce the partial or total occlusion of the artery by the collapse of arterial lining and to reduce the chance of the development of thrombosis and restenosis, an expandable, intraluminal prosthesis, one example of which is a stent, is implanted in the lumen to maintain the vascular patency.

Stents act as scaffoldings, functioning to physically hold open and, if desired, to expand the wall of the passageway. Typically, stents are capable of being compressed so that they can be inserted through small lumens via catheters and then expanded to a larger diameter once they are at the desired location. Examples in the patent literature disclosing stents that have been applied in PTCA procedures include U.S. Patent No. 4,733,665 issued to Palmaz, U.S. Patent No. 4,800,882 issued to Gianturco, and U.S. Patent No. 4,886,062 issued to Wiktor. Mechanical intervention via stents has reduced the rate of restenosis as compared to balloon angioplasty. Yet, restenosis is still a significant clinical problem with rates ranging from 20-40%. When restenosis does occur in the stented segment, its treatment can be challenging, as clinical options are more limited as compared to lesions that were treated solely with a balloon.

Stents are used not only for mechanical intervention but also as vehicles for providing biological therapy. Biological therapy can be achieved by medicating the stents. Medicated stents provide for the local administration of a therapeutic substance at the diseased site. In order to provide an efficacious concentration to the treated site, systemic administration of such medication often produces adverse or even toxic side effects for the patient. Local delivery is a preferred method of

treatment in that smaller total levels of medication are administered in comparison to systemic dosages, but are concentrated at a specific site. Local delivery thus produces fewer side effects and achieves more favorable results.

One proposed method for medicating stents included use of a heparin-coated metallic stent, whereby a heparin coating was ionically or covalently bonded to the stent. Significant disadvantages associated with the aforementioned method include loss of the therapeutic substance from the body of the stent during delivery and expansion of the stent as well as lack of control of the release rate of the substance from the stent.

Another proposed method of medicating stents involved the use of a polymeric carrier coated onto the surface of the stent. A composition including a solvent, a polymer dissolved in the solvent, and a therapeutic substance dispersed in the blend is applied to the stent by immersing the stent in the composition or by spraying the composition onto the stent. The solvent is allowed to evaporate, leaving on the stent strut surfaces a coating of the polymer and the therapeutic substance impregnated in the polymer.

Depending on the physiological mechanism targeted, the therapeutic substance may be required to be released at an efficacious concentration for an extended duration of time. Increasing the quantity of the therapeutic substance in the polymeric coating can lead to poor coating mechanical properties, inadequate coating adhesion, and overly rapid rate of release. Increasing the quantity of the polymeric compound by producing a thicker coating can perturb the geometrical

and mechanical functionality of the stent as well as limit the procedures for which the stent can be used.

It is desirable to increase the residence time of a substance at the site of implantation, at a therapeutically useful concentration, without needing to add a greater percentage of the therapeutic substance to the polymeric coating and without needing to apply a significantly thicker coating.

SUMMARY OF THE INVENTION

In accordance with one embodiment of the present invention, a method of forming a coating for a medical device, such as a stent, carrying an agent is provided. The method includes applying a first composition including a polymer to at least a portion of a medical device to form a first polymeric coating. The polymer has a solubility parameter not greater than approximately 11.5 $(cal/cm^3)^{1/2}$. The first polymeric coating reduces the rate of release of the agent from the medical device. In some embodiments of the method, the polymer of the first coating additionally has an equilibrium water absorption factor of less than about 5% by weight under physiologic conditions.

Also provided is a composition for forming a coating on a medical device. The composition includes a solvent and a hydrophobic polymer dissolved in the solvent. The hydrophobic polymer has an equilibrium water absorption factor of less than about 5% by weight under physiological conditions. In some embodiments, the hydrophobic polymer additionally has a solubility parameter not greater than approximately 11.5 $(cal/cm^3)^{1/2}$.

An implantable medical device for carrying a therapeutic agent is also provided. The device includes a first coating including a polymeric material. The polymeric material has a solubility parameter not greater than approximately 11.5 $(cal/cm^3)^{1/2}$. The first coating reduces the rate of release of the agent. In some embodiments, the polymeric material additionally has an equilibrium water absorption factor of less than about 5% by weight under physiologic conditions.

Polymeric material suitable for use in the first coating of the present invention include hydrophobic and non-polar polymers such as, but not limited to, polytetrafluoroethylene, perfluoro elastomers, amorphous fluoropolymer, ethylene-tetrafluoroethylene copolymer, fluoroethylene-alkyl vinyl ether copolymer, polyhexafluoropropylene, low density linear polyethylenes having high molecular weights, ethylene-olefin copolymers, atactic polypropylene, polyisobutene, polybutylenes, styrene-ethylene-styrene block copolymers, styrene-butylene-styrene block copolymers, styrene-ethylene/butylene-styrene block copolymers, styrene-butadiene-styrene block copolymers, ethylene-anhydride copolymers, ethylene vinyl acetate copolymers, ethylene-acrylic acid copolymers, ethylene methacrylic acid copolymers, polyurethanes with a polydimethylsiloxane soft segment, and cross-linked silicone elastomers.

The medical device can be, for example, a balloon-expandable stent, a self-expandable stent, a graft, a stent graft. The medical device can include cavities containing an active ingredient for the release of the active ingredient when the device is implanted. Alternatively, the device can include a reservoir coating carrying an active ingredient.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A illustrates a fluid on a solid substrate having a contact angle Φ_1;

Figure 1B illustrates a fluid on a solid substrate having a contact angle Φ_2;

Figure 2A illustrates a first coating deposited over an implantable medical substrate in accordance with one embodiment of the present invention;

Figure 2B illustrates a first coating deposited over an implantable medical substrate in accordance with another embodiment of the present invention; and

Figure 2C illustrates a pair of coatings deposited over an implantable medical substrate in accordance with yet another embodiment of the present invention.

DETAILED DESCRIPTION OF THE EMBODIMENTS

Composition for Forming an Optional Primer Layer

The presence of an active ingredient in a polymeric matrix can interfere with the ability of the matrix to adhere effectively to the surface of the device. Increasing the quantity of the active ingredient reduces the effectiveness of the adhesion. High drug loadings of, for example, 10-40% by weight in the coating can hinder the retention of the coating on the surface of the device. A primer layer can serve as a functionally useful intermediary layer between the surface of the device and an active ingredient-containing or reservoir coating. The primer layer provides an adhesive tie between the reservoir coating and the device -- which, in

effect, would also allow for the quantity of the active ingredient in the reservoir coating to be increased without compromising the ability of the reservoir coating to be effectively contained on the device during delivery and, if applicable, expansion of the device.

The embodiments of the composition for an optional primer layer are prepared by conventional methods wherein all components are combined, then blended. More particularly, in accordance with one embodiment, a predetermined amount of a polymer or a prepolymer is added to a predetermined amount of a solvent or a combination of solvents. The mixture can be prepared at ambient pressure and under anhydrous atmosphere. Heating and stirring and/or mixing can be employed to effect dissolution of the polymer into the solvent.

"Polymer," "poly," and "polymeric" are defined as compounds that are the product of a polymerization reaction and are inclusive of homopolymers, copolymers, terpolymers etc., including random, alternating, block, and graft variations thereof. The polymers should have a high capacity of adherence to the surface of an implantable device, such as a metallic surface of a stent.

Representative examples of suitable polymeric materials include, but are not limited to, polyisocyanates, such as triisocyanurate and polyisocyanate polyether polyurethanes based on diphenylmethane diisocyanate; acrylates, such as copolymers of ethyl acrylate and methacrylic acid; titanates, such as tetra-iso-propyl titanate and tetra-n-butyl titanate; zirconates, such as n-propyl zirconate and n-butyl zirconate; silane coupling agents, such as 3-aminopropyltriethoxysilane and

(3-glydidoxypropyl) methyldiethoxysilane; high amine content polymers, such as polyethyleneamine, polyallylamine, and polylysine; polymers with a high content of hydrogen bonding groups, such as polyethylene-co-polyvinyl alcohol, ethylene vinyl acetate, and melamine formaldehydes; and unsaturated polymers and prepolymers, such as polycaprolactone diacrylates, polyacrylates with at least two acrylate groups, and polyacrylated polyurethanes. With the use of unsaturated prepolymers, a free radical or UV initiator can be added to the composition for the thermal or UV curing or cross-linking process, as is understood by one of ordinary skill in the art.

Biocompatible polymers can also be used for the primer material. The polymer can be bioabsorbable or biostable. Bioabsorbable polymers that could be used include poly(hydroxyvalerate), poly(L-lactic acid), polycaprolactone, poly(lactide-co-glycolide), poly(hydroxybutyrate), poly(hydroxybutyrate-co-valerate), polydioxanone, polyorthoester, polyanhydride, poly(glycolic acid), poly(D,L-lactic acid), poly(glycolic acid-co-trimethylene carbonate), polyphosphoester, polyphosphoester urethane, poly(amino acids), cyanoacrylates, poly(trimethylene carbonate), poly(iminocarbonate), copoly(ether-esters) (e.g. PEO/PLA), polyalkylene oxalates, polyphosphazenes and biomolecules such as fibrin, fibrinogen, cellulose, starch, collagen and hyaluronic acid. In addition, biostable polymers such as polyurethanes, silicones, and polyesters could be used. Other polymers could also be used if they can be dissolved and cured or polymerized on the stent such as polyolefins, polyisobutylene and ethylene-alphaolefin copolymers; acrylic polymers and copolymers; vinyl halide polymers

and copolymers, such as polyvinyl chloride; polyvinyl ethers, such as polyvinyl methyl ether; polyvinylidene halides, such as polyvinylidene fluoride and polyvinylidene chloride; polyacrylonitrile, polyvinyl ketones; polyvinyl aromatics, such as polystyrene; polyvinyl esters, such as polyvinyl acetate; copolymers of vinyl monomers with each other and olefins, such as ethylene-methyl methacrylate copolymers, acrylonitrile-styrene copolymers, ABS resins, and ethylene-vinyl acetate copolymers; polyamides, such as Nylon 66 and polycaprolactam; alkyd resins; polycarbonates; polyoxymethylenes; polyimides; polyethers; epoxy resins; polyurethanes; rayon; rayon-triacetate; cellulose, cellulose acetate, cellulose butyrate; cellulose acetate butyrate; cellophane; cellulose nitrate; cellulose propionate; cellulose ethers; and carboxymethyl cellulose.

Ethylene vinyl alcohol is a very suitable choice of polymer for the primer layer. The copolymer possesses good adhesive qualities to the surface of a stent, particularly stainless steel surfaces, and has illustrated the ability to expand with a stent without any significant detachment of the copolymer from the surface of the stent. Ethylene vinyl alcohol copolymer, commonly known by the generic name EVOH or by the trade name EVAL, refers to copolymers comprising residues of both ethylene and vinyl alcohol monomers. One of ordinary skill in the art understands that ethylene vinyl alcohol copolymer may also be a terpolymer so as to include small amounts of additional monomers, for example less than about five (5) mole percentage of styrenes, propylene, or other suitable monomers. In a useful embodiment, the copolymer comprises a mole percent of ethylene of from about 27% to about 48%. Ethylene vinyl alcohol copolymers are available commercially

from companies such as Aldrich Chemical Company, Milwaukee, Wis., or EVAL Company of America, Lisle, IL, or can be prepared by conventional polymerization procedures that are well known to one of ordinary skill in the art.

The solvent should be compatible with the polymer and should be capable of placing the polymer into solution at the concentration desired. Particularly useful solvents should also be able to expand the chains of the polymer for maximum interaction with the surface of the device, such as a metallic surface of a stent. Examples of suitable solvents include, but are not limited to, dimethylsulfoxide (DMSO), chloroform, acetone, water (buffered saline), xylene, acetone, methanol, ethanol, 1-propanol, tetrahydrofuran, 1-butanone, dimethylformamide, dimethylacetamide, cyclohexanone, ethyl acetate, methylethylketone, propylene glycol monomethylether, isopropanol, N-methyl pyrrolidinone, toluene and mixtures thereof.

By way of example, and not limitation, the polymer can comprise from about 0.1% to about 35%, more narrowly from about 2% to about 20% by weight of the total weight of the composition, and the solvent can comprise from about 65% to about 99.9%, more narrowly from about 80% to about 98% by weight of the total weight of the composition. A specific weight ratio is dependent on factors such as the material from which the implantable device is made, the geometrical structure of the device, the choice of polymer-solvent combination, and the method of application.

In accordance with another embodiment, a fluid can be added to the composition to enhance the wetting of the primer composition for a more uniform coating application. To enhance the wetting of the composition, a suitable fluid typically has a high capillary permeation. Capillary permeation or wetting is the movement of a fluid on a solid substrate driven by interfacial energetics. Capillary permeation is quantitated by a contact angle, defined as an angle at the tangent of a droplet in a fluid phase that has taken an equilibrium shape on a solid surface. A low contact angle indicates a higher wetting liquid. A suitably high capillary permeation corresponds to a contact angle less than about 90°. Figure 1A illustrates a fluid droplet 10A on a solid substrate 12, for example a stainless steel surface. Fluid droplet 10A has a high capillary permeation that corresponds to a contact angle Φ_1, which is less than about 90°. In contrast, Figure 1B illustrates a fluid droplet 10B on solid substrate 12, having a low capillary permeation that corresponds to a contact angle Φ_2, which is greater than about 90°. The wetting fluid, typically, should have a viscosity not greater than about 50 centipoise, narrowly about 0.3 to about 5 centipoise, more narrowly about 0.4 to about 2.5 centipoise. The wetting fluid, accordingly, when added to the composition, reduces the viscosity of composition.

The wetting fluid should be compatible with the polymer and the solvent and should not precipitate the polymer. The wetting fluid can also act as the solvent. Useful examples of the wetting fluid include, but are not limited to, tetrahydrofuran (THF), dimethylformamide (DMF), 1-butanol, n-butyl acetate, dimethyl acetamide (DMAC), and mixtures and combinations thereof. By way of

example and not limitation, the polymer can comprise from about 0.1% to about 35%, more narrowly from about 2% to about 20% by weight of the total weight of the composition; the solvent can comprise from about 19.9% to about 98.9%, more narrowly from about 58% to about 84% by weight of the total weight of the composition; and the wetting fluid can comprise from about 1% to about 80%, more narrowly from about 5% to about 40% by weight of the total weight of the composition. The specific weight ratio of the wetting fluid depends on the type of polymer, solvent and wetting fluid employed as wells as the weight ratio of the polymer and the solvent.

Composition for Forming an Active Ingredient-Containing Coating

The embodiments of the composition for an active ingredient-containing or reservoir coating are prepared by conventional methods wherein all components are combined, then blended. More particularly, in accordance with one embodiment, a predetermined amount of a polymeric compound is added to a predetermined amount of a compatible solvent. The polymeric compound can be added to the solvent at ambient pressure and under anhydrous atmosphere. If necessary, gentle heating and stirring and/or mixing can be employed to effect dissolution of the polymer into the solvent, for example 12 hours in a water bath at about 60° C.

Sufficient amounts of an active ingredient are dispersed in the blended composition of the polymer and the solvent. The polymer can comprise from about 0.1% to about 35%, more narrowly from about 2% to about 20% by weight of the

total weight of the composition, the solvent can comprise from about 59.9% to about 99.8%, more narrowly from about 79% to about 89% by weight of the total weight of the composition, and the active ingredient can comprise from about 0.1% to about 40%, more narrowly from about 1% to about 9% by weight of the total weight of the composition. More than 9% by weight of the active ingredient could adversely affect characteristics that are desirable in the polymeric coating, such as adhesion of the coating to the device. With the use of the optional primer layer, weight ratios of more than 9% for the active ingredient are achievable without compromising the effectiveness of the adhesion. Selection of a specific weight ratio of the polymer and solvent is dependent on factors such as, but not limited to, the material from which the device is made, the geometrical structure of the device, and the type and amount of the active ingredient employed.

Optionally, a second solvent, such as tetrahydrofuran (THF) or dimethylformamide (DMF), can be used to improve the solubility of an active ingredient in the composition. The second solvent can be added to the composition or the active ingredient can be added to the second solvent prior to admixture with the blend. In this embodiment, the polymer can comprise from about 0.1% to about 35%, more narrowly from about 2% to about 20% by weight of the total weight of the composition, the solvent can comprise from about 19.8% to about 98.8%, more narrowly from about 49% to about 79% by weight of the total weight of the composition; the second solvent can comprise from about 1% to about 80%, more narrowly from about 5% to about 40% by weight of the total weight of the composition; and the active ingredient can comprise from about 0.1% to about

40%, more narrowly from about 1% to about 9% by weight of the total weight of the composition. Selection of a specific weight ratio of the polymer, the solvent, and the second solvent is dependent on factors such as, but not limited to, the material from which the implantable device is made, the geometrical structure of the device, and the type and amount of the active ingredient employed. The particular weight percentage of the active ingredient mixed within the composition depends on factors such as duration of the release, cumulative amount of release, and release rate that is desired.

The active ingredient should be in true solution or saturated in the blended composition. If the active ingredient is not completely soluble in the composition, operations including mixing, stirring, and/or agitation can be employed to effect homogeneity of the residues. The active ingredient can also be first added to the second solvent prior to admixing with the composition. The active ingredient may be added so that the dispersion is in fine particles. The mixing of the active ingredient can be conducted in an anhydrous atmosphere, at ambient pressure, and at room temperature such that supersaturating the active ingredient is not achieved.

The active ingredient may be any substance capable of exerting a therapeutic or prophylactic effect in the practice of the present invention. Examples of such active ingredients include antiproliferative, antineoplastic, antiinflammatory, antiplatelet, anticoagulant, antifibrin, antithrombin, antimitotic, antibiotic, and antioxidant substances as well as combinations thereof. A suitable example of an antiproliferative substance is actinomycin D, or derivatives and analogs thereof (manufactured by Sigma-Aldrich 1001 West Saint Paul Avenue,

Milwaukee, WI 53233; or COSMEGEN available from Merck). Synonyms of actinomycin D include dactinomycin, actinomycin IV, actinomycin I_1, actinomycin X_1, and actinomycin C_1. Examples of suitable antineoplastics include paclitaxel and docetaxel. Examples of suitable antiplatelets, anticoagulants, antifibrins, and antithrombins include sodium heparin, low molecular weight heparin, hirudin, argatroban, forskolin, vapiprost, prostacyclin and prostacyclin analogs, dextran, D-phe-pro-arg-chloromethylketone (synthetic antithrombin), dipyridamole, glycoprotein IIb/IIIa platelet membrane receptor antagonist, recombinant hirudin, thrombin inhibitor (available from Biogen), and 7E-3B® (an antiplatelet drug from Centocore). Examples of suitable antimitotic agents include methotrexate, azathioprine, vincristine, vinblastine, fluorouracil, adriamycin, and mutamycin. Examples of suitable cytostatic or antiproliferative agents include angiopeptin (a somatostatin analog from Ibsen), angiotensin converting enzyme inhibitors such as CAPTOPRIL (available from Squibb), CILAZAPRIL (available from Hoffman-LaRoche), or LISINOPRIL (available from Merck); calcium channel blockers (such as Nifedipine), colchicine, fibroblast growth factor (FGF) antagonists, histamine antagonist, LOVASTATIN (an inhibitor of HMG-CoA reductase, a cholesterol lowering drug from Merck), monoclonal antibodies (such as PDGF receptors), nitroprusside, phosphodiesterase inhibitors, prostaglandin inhibitor (available form Glazo), Seramin (a PDGF antagonist), serotonin blockers, thioprotease inhibitors, triazolopyrimidine (a PDGF antagonist), and nitric oxide. Other therapeutic substances or agents that may be appropriate include alpha-interferon; genetically engineered epithelial cells; dexamethasone; rapamycin; estradiol; clobetasol propionate; cisplatin; and carboplatin. Exposure of the

composition to the active ingredient should not adversely alter the active ingredient's composition or characteristic. Accordingly, the particular active ingredient is selected for compatibility with the blended composition.

The dosage or concentration of the active ingredient required to produce a therapeutic effect should be less than the level at which the active ingredient produces toxic effects and greater than the level at which non-therapeutic results are obtained. The dosage or concentration of the active ingredient required to inhibit the desired cellular activity of the vascular region, for example, can depend upon factors such as the particular circumstances of the patient; the nature of the trauma; the nature of the therapy desired; the time over which the ingredient administered resides at the vascular site; and if other bioactive substances are employed, the nature and type of the substance or combination of substances. Therapeutically effective dosages can be determined empirically, for example by infusing vessels from suitable animal model systems and using immunohistochemical, fluorescent or electron microscopy methods to detect the agent and its effects, or by conducting suitable in vitro studies. Standard pharmacological test procedures to determine dosages are understood by one of ordinary skill in the art.

The polymer chosen should be biocompatible so as not to cause any adverse response. The solvent chosen should be capable of placing the polymer into solution at the concentration desired. Representative examples of biocompatible polymers as well as of suitable solvents include those provided above with reference to the primer composition. With the use of a low ethylene content, e.g.,

29 mol%, ethylene vinyl alcohol, for example, a suitable solvent is iso-propylalcohol (IPA) admixed with water e.g., from about 40% to about 60% by weight IPA. If an optional primer layer is used, the choice of polymer for the reservoir coating can be the same as that selected for the primer so as to eliminate any interfacial incompatibilities.

Composition for Forming the Rate-Reducing Membrane

If it is desired to increase the rate at which an active ingredient diffuses through a membrane, the membrane should be made of a polymer in which the active ingredient readily dissolves. By contrast, if it is desired to decrease the rate at which an active ingredient diffuses through a membrane, the membrane should be made of a polymer in which the active ingredient is less soluble. The purpose of the rate-reducing membrane of the present invention is to decrease the rate of release of an underlying active ingredient. Accordingly, the polymer for forming the rate-reducing membrane should be selected such that the active ingredient may not readily dissolve therein.

Polar substances are substances that have a dipole moment μ greater than 0 Debye. As a general rule, polar substances dissolve well in other polar substances, such as water. Accordingly, polar substances can be broadly categorized as "hydrophilic." Polar substances typically dissolve less readily in non-polar substances, which can be broadly categorized as "hydrophobic." The syllogism follows that a polar active ingredient will not readily dissolve in a hydrophobic polymer. Accordingly, a polymeric membrane that is hydrophobic may be

employed to reduce the rate at which a polar active ingredient is released from an implantable device, such as a stent.

One method of defining the hydrophobicity of a polymer is by the solubility parameter of the polymer. The solubility parameter is represented by Equation 1:

$$\delta = (\Delta E/V)^{1/2} \qquad \text{(Equation 1)}$$

where δ = solubility parameter $((\text{cal/cm}^3)^{1/2})$
ΔE = energy of vaporization (cal)
V = molar volume (cm^3)

("Polymer Handbook", 2nd Ed., Brandrup J. and EH Immergut, ed., Wiley-Interscience, John Wiley & Sons, N.Y. (1975)). Because polymers are typically non-volatile and thus cannot be vaporized without decomposition, the solubility parameter is measured indirectly. Briefly, solvents in which a polymer dissolves without a change in heat or volume are identified. The solubility parameter of the polymer is then defined to be the same as the solubility parameters of the identified solvents.

As a general rule, the value of the solubility parameter δ is inversely proportional to the degree of hydrophobicity of a polymer. Polymers that are very hydrophobic may have a low solubility parameter value. This general proposition is particularly applicable for polymers having a glass transition temperature below physiological temperature. A polymer that is sufficiently hydrophobic for use in the rate-limiting membrane of the present invention can have a solubility parameter

not more than about 11.5 $(cal/cm^3)^{1/2}$, more narrowly not more than about 10 $(cal/cm^3)^{1/2}$, even more narrowly not more than 8.5 $(cal/cm^3)^{1/2}$.

Table 1 illustrates the solubility parameters of various polymers.

Table 1

Polymer	Solubility Parameter $(cal/cm^3)^{1/2}$
polytetrafluoroethylene	6.2
polydimethylsiloxane	7.3-7.62
polyethylene	7.7-8.79
polybutylmethacrylate	8.3-8.8
polypropylene	9.2-9.4
ethyl cellulose	10.3
polyvinyl acetate	9.4-11.0

Another method of defining the hydrophobicity of a polymer is by the equilibrium moisture absorption factor. The equilibrium moisture absorption factor is represented by Equation 2:

$$MAF = (W_w/W_p + W_w) \times 100 \qquad \text{(Equation 2)}$$

where MAF = equilibrium moisture absorption factor (%)
W_w = weight of water taken up by the polymer when immersed in water or exposed to physiologic conditions
W_p = weight of the polymer

Generally, the less water absorbed by a polymer, and thus the lower the equilibrium moisture absorption factor, the better the polymer functions as a diffusion barrier for a polar active ingredient. Upon absorption of water, a polymer can swell and the spaces between polymer chains can enlarge, allowing an active ingredient to diffuse more easily through the polymer. In addition, absorbed water provides an otherwise non-polar polymer with polar water groups. These polar regions more readily dissolve a polar active ingredient via hydrogen bonding interactions, thereby allowing the active ingredient to diffuse through the otherwise non-polar polymer more quickly. Thus, a polymer that is sufficiently hydrophobic for use in the rate-limiting membrane of the present invention should have an equilibrium moisture absorption factor of less than about 5% by weight, more narrowly less than about 2%. Representative examples of such polymers include polytetrafluoroethylene, perfluoro elastomers, fluoropolymers such as polyvinylidene fluoride, ethylene-tetrafluoroethylene copolymer, fluoroethylene-alkyl vinyl ether copolymer, polyhexafluoropropylene, low density linear polyethylenes having high molecular weights, ethylene-olefin copolymers, atactic polypropylene, polyisobutene, polybutylenes, polybutenes, styrene-ethylene-styrene block copolymers, styrene-butylene-styrene block copolymers, styrene-ethylene/butylene-styrene block copolymers, styrene-butadiene-styrene block copolymers, ethylene-anhydride copolymers, ethylene vinyl acetate copolymers, polybutylmethacrylate, ethylene-acrylic acid copolymers of low acrylic acid content, ethylene methacrylic acid copolymers of low methacrylic acid content, ethylene vinyl alcohol copolymers with an ethylene content greater than 48 mole percent, and cross-linked silicone elastomers.

In addition to having a solubility parameter not more than about 11.5 $(cal/cm^3)^{1/2}$ and/or an equilibrium moisture absorption factor of less than about 5% by weight, the selected polymer should be biocompatible. The polymer should also be capable of being placed into solution at a desired concentration by a selected solvent, such as a non-polar solvent so as to prevent dissolution of the polar active ingredient with the non-polar solvent. Prevention of dissolution of the active ingredient during the coating process of the rate-limiting layer significantly reduces or eliminates the migration or leaching of the active component out from the underlying reservoir layer or device. Accordingly, the quantity of active ingredient will not be reduced during the application of the rate-limiting layer.

Fluoropolymers are a suitable choice for the barrier layer composition. For example, the solubility parameter of polytetrafluoroethylene is about 6.2 $(cal/cm^3)^{1/2}$, and the equilibrium moisture absorption factor is about 0.01%. Solution processing of fluoropolymers is possible, particularly the low crystallinity varieties such as CYTOP available from Asahi Glass and TEFLON AF available from DuPont. Solutions of up to about 15% (wt/wt) are possible in perfluoro solvents, such as FC-75 (available from 3M under the brand name FLUORINERT), which are non-polar, low boiling solvents. Such volatility allows the solvent to be easily and quickly evaporated following the application of the polymer-solvent solution to the medical device.

Another particularly suitable choice of polymer for the barrier layer composition is styrene-ethylene/butylene-styrene block copolymer. The solubility parameter of this material lies in the range of from about 7.7 $(cal/cm^3)^{1/2}$ to about

10.3 $(cal/cm^3)^{1/2}$, and the equilibrium moisture absorption factor is less than about 1%. Styrene-ethylene/butylene-styrene block copolymer, e.g., Kraton G-series, can be dissolved in non-polar solvents such as, but not limited to, toluene, xylene, and decalin.

Still other suitable choices of polymers for the rate-limiting membrane include, but are not limited to, ethylene-anhydride copolymers; ethylene vinyl acetate copolymers having, for example, a mol % of vinyl acetate of from about 9% to about 25%; and ethylene-acrylic acid copolymers having, for example, a mol % of acrylic acid of from about 2% to about 25%. The ethylene-anhydride copolymer available from Bynel adheres well to EVAL and thus would function well as a topcoat over a reservoir layer made from EVAL. The copolymer can be dissolved in organic solvents, such as dimethylsulfoxide and dimethylacetamide. Ethylene vinyl acetate polymers can be dissolved in organic solvents, such as toluene and n-butyl acetate. Ethylene-acrylic acid copolymers can be dissolved in organic solvents, such as methanol, isopropyl alcohol, and dimethylsulfoxide.

Yet another suitable choice of polymer for the rate-limiting membrane composition is a cross-linked silicone elastomer. Such substances have a solubility parameter in the range of about 7.3 $(cal/cm^3)^{1/2}$ to about 7.6 $(cal/cm^3)^{1/2}$, and an equilibrium moisture absorption factor of less than about 0.5%. Loose silicone and silicone with very low cross-linking are thought to cause an inflammatory biological response. However, it is believed that a thoroughly cross-linked silicone elastomer, having low levels of leachable silicone polymer and oligomer, is an essentially non-inflammatory substance. Silicone elastomers, such as Nusil MED-

4750, MED-4755, or MED2-6640, having high tensile strengths, for example between 1200 psi and 1500 psi, will likely have the best durability during crimping, delivery, and expansion of a stent as well as good adhesion to a reservoir layer, e.g., EVAL or the surface of a medical device.

The embodiments of the composition for a rate-reducing membrane or diffusion barrier layer are prepared by methods wherein all components are combined, then blended. More particularly, in accordance with one embodiment, a predetermined amount of a polymeric compound is added to a predetermined amount of a compatible solvent. The selected solvent should be capable of placing the polymer into solution at the concentration desired.

The polymeric compound can be added to the solvent at ambient pressure and under anhydrous atmosphere. If necessary, gentle heating and stirring and/or mixing can be employed to effect dissolution of the polymer into the solvent, for example 12 hours in a water bath at about 60° C. The polymer can comprise from about 0.1% to about 35%, more narrowly from about 2% to about 20% by weight of the total weight of the composition, and the solvent can comprise from about 65% to about 99.9%, more narrowly from about 80% to about 98% by weight of the total weight of the composition. Selection of a specific weight ratio of the polymer and solvent is dependent on factors such as, but not limited to, the type of polymer and solvent employed, the type of underlying reservoir layer, and the method of application. Optionally, one of the aforementioned wetting fluids can also be added to the blend.

Examples of the Device

The device or prosthesis used in conjunction with the above-described compositions may be any suitable medical substrate that can be implanted in a human or veterinary patient. Examples of such implantable devices include self-expandable stents, balloon-expandable stents, stent-grafts, grafts, artificial heart valves, cerebrospinal fluid shunts, and pacemaker electrodes. The underlying structure of the device can be virtually any design. The device can be made of a metallic material or an alloy such as, but not limited to, cobalt chromium alloy (ELGILOY), stainless steel (316L), high nitrogen stainless steel, e.g., BIODUR 108, cobalt chrome alloy L-605, "MP35N," "MP20N," ELASTINITE (Nitinol), tantalum, nickel-titanium alloy, platinum-iridium alloy, gold, magnesium, or combinations thereof. "MP35N" and "MP20N" are trade names for alloys of cobalt, nickel, chromium and molybdenum available from standard Press Steel Co., Jenkintown, PA. "MP35N" consists of 35% cobalt, 35% nickel, 20% chromium, and 10% molybdenum. "MP20N" consists of 50% cobalt, 20% nickel, 20% chromium, and 10% molybdenum. Devices made from bioabsorbable or biostable polymers could also be used with the embodiments of the present invention.

It should be noted that the rate-reducing membrane or diffusion barrier layer in accordance with various embodiments of the present invention can be used without the active ingredient-containing coating. In such embodiments, the device may include cavities or micro-pores for containing the active ingredient such that the diffusion barrier layer is disposed over the cavities. The device can be formed by sintering the stent material from metallic particles, filaments, fibers or other

materials. The device can be formed from a sintered wire that is coiled or otherwise formed into a device. The prosthesis can also be formed from a sintered cylindrical tube or sintered metal sheet which can be laser cut or chemical etched into an expandable stent structure. Formation of cavities via a sintering process is described in U.S. Patent No. 5,843,172 to Yan. By way of another example, the surface of the device can be exposed to an etchant or a laser discharge to form cavities of selected dimensional specification.

Methods For Applying the Compositions to the Device

To form the optional primer layer and/or the active ingredient-containing coating on a surface of the device or prosthesis, the surface of the device should be clean and free from contaminants that may be introduced during manufacturing. However, the surface of the prosthesis requires no particular surface treatment to retain the applied coating. Application of the composition can be by any conventional method, such as by spraying the composition onto the prosthesis or by immersing the prosthesis in the composition. Operations such as wiping, centrifugation, blowing, or other web-clearing acts can also be performed to achieve a more uniform coating. Briefly, wiping refers to physical removal of excess coating from the surface of the stent; centrifugation refers to rapid rotation of the stent about an axis of rotation; and blowing refers to application of air at a selected pressure to the deposited coating. Any excess coating can also be vacuumed off the surface of the device. The addition of a wetting fluid leads to a consistent application of the composition which also causes the coating to be uniformly deposited on the surface of the prosthesis.

With the use of the thermoplastic polymers for the primer, such as ethylene vinyl alcohol copolymer, polycaprolactone, poly(lactide-co-glycolide), poly(hydroxybutyrate), etc., the deposited primer composition should be exposed to a heat treatment at a temperature range greater than about the glass transition temperature (T_g) and less than about the melting temperature (T_m) of the selected polymer. Unexpected results have been discovered with treatment of the composition under this temperature range, specifically strong adhesion or bonding of the coating to the metallic surface of a stent. The device should be exposed to the heat treatment for any suitable duration of time that would allow for the formation of the primer coating on the surface of the device as well as for the evaporation of the solvent or combination of solvent and wetting fluid. It is understood that essentially all of the solvent and the wetting fluid will be removed from the composition, but traces or residues may remain blended with the polymer.

Table 2 lists the T_g and T_m for some of the polymers used in the embodiments of the present invention. T_g and T_m of polymers are attainable by one of ordinary skill in the art. The cited exemplary temperature and time for exposure are provided by way of illustration and are not meant to be limiting.

Table 2

Polymer	T_g (°C)	T_m (°C)	Exemplary Temperature (°C)	Exemplary Duration of Time For Heating
EVAL	55	165	140	4 hours
polycaprolactone	-60	60	50	2 hours
ethylene vinyl acetate (e.g., 33% vinyl acetate content)	36	63	45	2 hours
Polyvinyl alcohol	75-85*	200-220*	165	2 hours

* Exact temperature depends on the degree of hydrolysis which is also known as the amount of residual acetate.

With the use of one of the aforementioned thermoset primer polymers, the use of initiators may be required. By way of example, epoxy systems consisting of diglycidyl ether of bisphenol A resins can be cured with amine curatives, thermoset polyurethane prepolymers can cured with polyols, polyamines, or water (moisture), and acrylated urethane can be cured with UV light. If baked, the temperature can be above the T_g of the selected polymer.

With the use of the inorganic primer polymers, such as silanes, titanates, and zirconates, the solvent is allowed to evaporate.

The composition containing the active ingredient can be applied to a designated region of the primer coating or the surface of the device. The solvent(s) or the combination of solvent(s) and the wetting fluid is removed from the composition by allowing the solvent(s) or combination of the solvent(s) and the wetting fluid to evaporate. The evaporation can be induced by heating the device at a predetermined temperature for a predetermined period of time. For example,

the device can be heated at a temperature of about 60° C for about 12 hours to about 24 hours. The heating can be conducted in an anhydrous atmosphere and at ambient pressure and should not exceed the temperature which would adversely affect the active ingredient. The heating can, alternatively, be conducted under a vacuum condition. It is understood that essentially all of the solvent and the wetting fluid will be removed from the composition, but traces or residues may remain blended with the polymer.

The diffusion barrier layer can be formed on a designated region of the active ingredient-containing coating subsequent to the evaporation of the solvent(s) or solvent(s)/wetting fluid and the drying of the polymer for the active ingredient-containing coating. Alternatively, in embodiments in which a polymeric reservoir coating is not employed, the rate-reducing membrane may be formed directly over active-ingredient containing cavities within the surface of the prosthesis. The diffusion barrier layer can be applied by spraying the composition onto the device or immersing the device in the composition, then drying the polymer. The above-described processes can be similarly repeated for the formation of the diffusion barrier layer.

Coating

Some of the various embodiments of the present invention are illustrated by Figures 2A, 2B, and 2C. The Figures have not been drawn to scale, and the thickness of the various layers have been over or under emphasized for illustrative purposes.

Referring to Figure 2A, a body of a medical substrate 20, such as a stent, is illustrated having a surface 22. Medical substrate 20 includes cavities or micro-pores 24 formed in the body for releasably containing an active ingredient, as illustrated by dotted region 26. A diffusion barrier layer or rate-reducing membrane 28 is disposed on surface 22 of medical substrate 20, covering cavities 24. Diffusion barrier layer 28 functions to reduce the rate of release of an active ingredient from medical substrate 20.

Referring to Figure 2B, medical substrate 20 is illustrated having a primer layer 30 formed on surface 22. An active ingredient-containing or reservoir coating 32 is deposited on primer layer 30. Primer layer 30 serves as an intermediary layer for increasing the adhesion between reservoir coating 32 and surface 22. Increasing the amount of active ingredient admixed within the polymer diminishes the adhesiveness of reservoir layer 32 to surface 22. Accordingly, using an active ingredient-free polymer as an intermediary primer layer 30 allows for a higher active ingredient content for reservoir layer 32. Diffusion barrier 28 is formed over at least a selected portion of reservoir layer 32. One of ordinary skill in the art can appreciate that diffusion barrier layer 28 can be deposited only on selected areas of reservoir layer 32 so as to provide a variety of selected release parameters. Such selected patterns may become particularly useful if a combination of active ingredients are used, each of which requires a different release parameter.

Figure 2C illustrates medical substrate 20 having a first reservoir layer 32A disposed on a selected portion of surface 22 of medical substrate 20. First reservoir

layer 32A contains a first active ingredient, e.g., actinomycin D. A second reservoir layer 32B can also be disposed on surface 22. Second reservoir layer 32B contains a second active ingredient, e.g., taxol. First and second reservoir layers 32A and 32B are covered by first and second diffusion barrier layers 28A and 28B, respectively. In accordance with one embodiment, the polymeric material from which diffusion barrier layer 28A is made can be different than the material from which diffusion barrier layer 28B is made. Accordingly, a wide array of release parameters can be obtained for any selected combination of active ingredients.

Diffusion barrier layer 28 can have any suitable thickness, as the thickness of diffusion barrier layer 28 is dependent on parameters such as, but not limited to, the desired rate of release and the procedure for which the stent will be used. Diffusion barrier layer 28 can have a thickness of about 0.1 to about 10 microns, more narrowly from about 0.25 to about 5 microns.

By way of example, and not limitation, the impregnated reservoir layer 32 can have a thickness of about 0.5 microns to about 1.5 microns. The particular thickness of reservoir layer 32 is based on the type of procedure for which medical substrate 20 is employed and the amount of the active ingredient to be delivered. The amount of the active ingredient to be included on the prosthesis can be further increased by applying a plurality of reservoir layers 32 on top of one another. The optional primer layer 30 can have any suitable thickness, examples of which can be in the range of about 0.1 to about 10 microns, more narrowly about 0.1 to about 2 microns.

Method of Use

In accordance with the above-described method, the active ingredient can be applied to a device, e.g., a stent, retained on the device during delivery and released at a desired control rate and for a predetermined duration of time at the site of implantation. A stent having the above-described coating layers is useful for a variety of medical procedures, including, by way of example, treatment of obstructions caused by tumors in bile ducts, esophagus, trachea/bronchi and other biological passageways. A stent having the above-described coating layers is particularly useful for treating occluded regions of blood vessels caused by abnormal or inappropriate migration and proliferation of smooth muscle cells, thrombosis, and restenosis. Stents may be placed in a wide array of blood vessels, both arteries and veins. Representative examples of sites include the iliac, renal, and coronary arteries.

Briefly, an angiogram is first performed to determine the appropriate positioning for stent therapy. Angiography is typically accomplished by injecting a radiopaque contrasting agent through a catheter inserted into an artery or vein as an x-ray is taken. A guidewire is then advanced through the lesion or proposed site of treatment. Over the guidewire is passed a delivery catheter, which allows a stent in its collapsed configuration to be inserted into the passageway. The delivery catheter is inserted either percutaneously, or by surgery, into the femoral artery, brachial artery, femoral vein, or brachial vein, and advanced into the appropriate blood vessel by steering the catheter through the vascular system under fluoroscopic guidance. A stent having the above-described coating layers may then

be expanded at the desired area of treatment. A post insertion angiogram may also be utilized to confirm appropriate positioning.

EXAMPLES

The embodiments of the invention will be illustrated by the following set forth prophetic examples which are being given by way of illustration only and not by way of limitation. All parameters and data are not to be construed to unduly limit the scope of the embodiments of the invention.

Example 1

A 13 mm, 316L stainless steel TETRA stent is primer coated by spraying with a 2% (w/w) solution of poly(ethylene-co-vinyl alcohol) (44 mole % ethylene) in dimethylacetamide. The solvent is removed by baking at 140°C for 1 hour. A solution of 2% (w/w) EVAL and 0.25% (w/w) actinomycin D in dimethylacetamide is spray coated onto the stent to a thickness that gives 25 μg of actinomycin D on the stent. The stent is then baked at 50°C for two hours. A hydrophobic release rate limiting membrane is formed by spraying the stent with a 2% (w/w) solution of polybutylmethacrylate in a 1/3 (w/w) mixture of ethyl acetate and cyclohexanone. A second two hour bake at 50°C is performed to remove the solvent.

Example 2

A 13 mm, 316L stainless steel TETRA stent is primer coated by spraying with a 2% (w/w) solution of poly(ethylene-co-vinyl alcohol) (44 mole % ethylene)

in dimethylacetamide. The solvent is removed by baking at 140°C for 1 hour. A solution of 2% (w/w) EVAL and 0.5% (w/w) paclitaxel in dimethylacetamide is spray coated onto the stent to a thickness that gives 50 µg of paclitaxel on the stent. The stent is then baked at 50°C for two hours. A hydrophobic release rate limiting membrane is formed by spraying on a 2% (w/w) solution of poly(ethylene-co-vinylacetate) (25 mole % acetate content) in a 1/1 (w/w) solution of toluene and n-butyl acetate. Another two hour bake at 50°C is performed to remove the solvent.

Example 3

A 13 mm, 316L stainless steel TETRA stent is primer coated by spraying with a 2% (w/w) solution of poly(ethylene-co-vinyl alcohol) (44 mole % ethylene) in dimethylacetamide. The solvent is removed by baking at 140°C for 1 hour. A solution of 2% (w/w) EVAL and 0.67% (w/w) clobetasol propionate in dimethylacetamide is spray coated onto the stent to a thickness that gives 150 µg of clobetasol propionate on the stent. The stent is then baked at 50°C for two hours. A hydrophobic release rate limiting membrane is formed by coating on a 5% (w/w) solution of Nusil MED3-6605 silicone dispersion in a 1/1 (w/w) of trichloroethylene and cyclohexane. This process is accomplished by placing the stent on a section of 0.070 inch OD stainless steel tubing. The coating is then applied to the stent via syringe. With the stent covered with fluid, it is pushed along the length of the tube with a short section of TEFLON tubing, while simultaneously rotating the stainless steel tube. The coating is left to air cure at ambient temperature for 18 hours.

While particular embodiments of the present invention have been shown and described, it will be obvious to those skilled in the art that changes and modifications can be made without departing from this invention in its broader aspects. For example, while much of the above discussion focuses on the reduction of the rate of diffusion of a polar active ingredient, one of ordinary skill in the art will understand that the diffusion barriers described are also applicable for use with non-polar active ingredients. Therefore, the appended claims are to encompass within their scope all such changes and modifications as fall within the true spirit and scope of this invention.

CLAIMS

What is claimed is:

1. A method of forming a coating for a medical device carrying an agent, comprising:

applying a first composition including a polymer to at least a portion of a medical device to form a first coating, said polymer having a solubility parameter not greater than approximately 11.5 $(cal/cm^3)^{1/2}$, and

wherein said first coating reduces the rate of release of said agent from said medical device.

2. A coating for a medical device produced in accordance with the method of Claim 1.

3. The method of Claim 1, wherein said medical device is a balloon expandable stent, a self-expandable stent, a stent-graft, or a graft.

4. The method of Claim 1, wherein said medical device is a metallic stent having cavities containing said agent for the release of said agent subsequent to the implantation of said stent in a mammalian lumen, and wherein said first coating is formed on the surface of said metallic stent and covering said cavities.

5. The method of Claim 1, wherein said agent is a polar substance.

6. The method of Claim 1, wherein said first coating is hydrophobic, and wherein said agent is a polar substance.

7. The method of Claim 1, wherein said first composition additionally includes a solvent capable of dissolving said polymer, said method additionally comprising evaporating said solvent to form said first coating.

8. The method of Claim 7, wherein said solvent is non-polar and capable of dissolving said polymer.

9. The method of Claim 7, wherein said solvent is non-polar and capable of dissolving the polymer but not the agent.

10. The method of Claim 1, wherein said agent is selected from a group of actinomycin D, docetaxel, paclitaxel, and rapamycin.

11. The method of Claim 1, wherein said polymer has an equilibrium water absorption factor of less than about 5% by weight under physiologic conditions.

12. The method of Claim 1, additionally comprising prior to said applying a first composition:

(a) applying a second composition including a solvent and a polymer to the surface of said medical device;

(b) evaporating said solvent of said second composition to form a second coating on the surface of said medical device;

(c) applying a third composition including a solvent, a polymer, and an agent on said second coating; and

(d) evaporating said solvent of said third composition to form a third coating containing said agent in said second coating,

wherein said first coating reduces the rate of release of said agent.

13. The method of Claim 1, additionally comprising prior to said applying a first composition:

(a) applying a second composition including a solvent, a polymer, and an agent to the surface of said medical device; and

(b) evaporating said solvent of said second composition to form a second coating containing said agent on the surface of said medical device,

wherein said first coating reduces the rate of release of said agent.

14. The method of Claim 1, wherein said polymer has a solubility parameter not greater than approximately 10 $(cal/cm^3)^{1/2}$.

15. The method of Claim 1, wherein said polymer has a solubility parameter not greater than approximately 8.5 $(cal/cm^3)^{1/2}$.

16. A composition for forming a coating on a medical device comprising:

(a) a solvent; and

(b) a hydrophobic polymer dissolved in said solvent, wherein said polymer has an equilibrium water absorption factor of less than about 5% by weight by weight under physiological conditions.

17. The composition of Claim 16, wherein said solvent is non-polar and capable of dissolving said polymer.

18. A polymeric coating produced by the evaporation of said solvent from said composition of Claim 16.

19. The composition of Claim 16, wherein said polymer is selected from a group of polytetrafluoroethylene, perfluoro elastomers, fluoropolymers, ethylene-tetrafluoroethylene copolymer, fluoroethylene-alkyl vinyl ether copolymer, polyhexafluoropropylene, low density linear polyethylenes having high molecular weights, ethylene-olefin copolymers, atactic polypropylene, polyisobutene, polybutylenes, styrene-ethylene-styrene block copolymers, styrene-butylene-styrene block copolymers, styrene-ethylene/butlene-styrene block copolymers, styrene-butadiene-styrene block copolymers, ethylene-anhydride

copolymers, ethylene vinyl acetate copolymers, ethylene-acrylic acid copolymers, ethylene methacrylic acid copolymers, polyurethanes with a polydimethylsiloxane soft segment, ethylene vinyl alcohol copolymers with an ethylene content greater than 48 mole percent, and cross-linked silicone elastomers.

20. The composition of Claim 16, wherein said medical device is a radially expandable stent.

21. The composition of Claim 16, wherein said coating formed from said composition is used for reducing the rate of release of a therapeutic agent from said medical device.

22. The composition of Claim 16, wherein said polymer has a solubility parameter not greater than approximately 11.5 $(cal/cm^3)^{1/2}$.

23. The composition of Claim 16, wherein said polymer has a solubility parameter not greater than approximately 8.5 $(cal/cm^3)^{1/2}$.

24. An implantable medical device for carrying a therapeutic agent, comprising:

a first coating including a polymeric material, said polymeric material having a solubility parameter not greater that approximately 11.5 $(cal/cm^3)^{1/2}$, wherein said first coating reduces the rate of release of said agent.

25. The device of Claim 24, wherein said polymeric material is hydrophobic and said therapeutic agent is polar.

26. The device of Claim 24, wherein said polymeric material is non-polar and said therapeutic agent is polar.

27. The device of Claim 24, additionally comprising:

(a) a second polymeric coating formed on the surface of said medical device; and

(b) a third polymeric coating including an agent formed on said second polymeric coating and beneath said first polymeric coating,

wherein said first polymeric coating reduces the rate of release of said agent.

28. The device of Claim 24, additionally comprising:

(a) a second polymeric coating including an agent formed on the surface of said medical device and beneath said first polymeric coating,

wherein said first polymeric coating reduces the rate of release of said agent.

29. The device of Claim 24, wherein said polymeric material is selected from a group of polytetrafluoroethylene, perfluoro elastomers, fluoropolymers, ethylene-tetrafluoroethylene copolymer, fluoroethylene-alkyl vinyl ether copolymer, polyhexafluoropropylene, low density linear polyethylenes having high

molecular weights, ethylene-olefin copolymers, atactic polypropylene, polyisobutene, polybutylenes, styrene-ethylene-styrene block copolymers, styrene-butylene-styrene block copolymers, styrene-ethylene/butlene-styrene block copolymers, styrene-butadiene-styrene block copolymers, ethylene-anhydride copolymers, ethylene vinyl acetate copolymers, ethylene-acrylic acid copolymers, ethylene methacrylic acid copolymers, polyurethanes with a polydimethylsiloxane soft segment, ethylene vinyl alcohol copolymers with an ethylene content greater than 48 mole percent, and cross-linked silicone elastomers.

30. The device of Claim 24, wherein said implantable medical device is a radially expandable metallic stent.

31. The device of Claim 30, wherein said metallic stent includes cavities containing said agent for the release of said agent subsequent to the implantation of said stent in a mammalian lumen, and wherein said first polymeric coating is formed on the surface of said metallic stent and covering said cavities.

32. The device of Claim 24, wherein said polymeric material has an equilibrium water absorption factor of less than about 5% by weight under physiologic conditions.

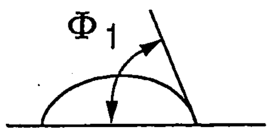

Figure 1A

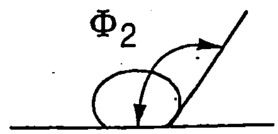

Figure 1B

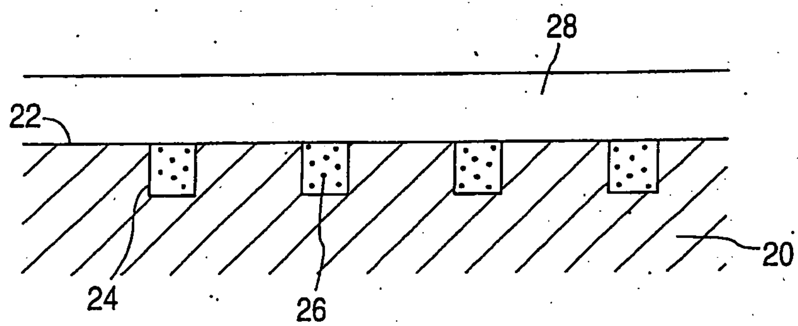

Figure 2A

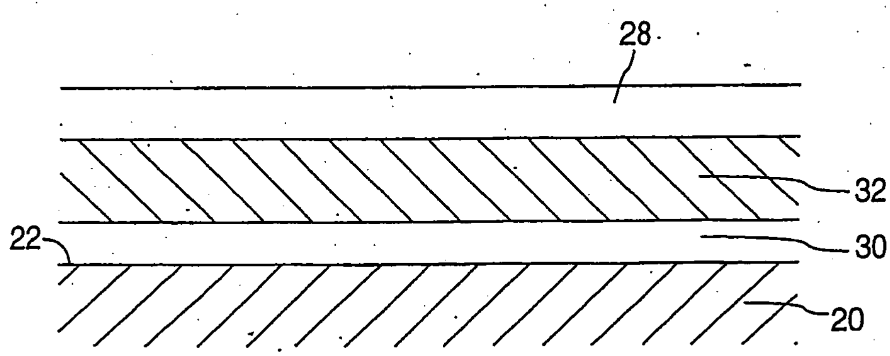

Figure 2B

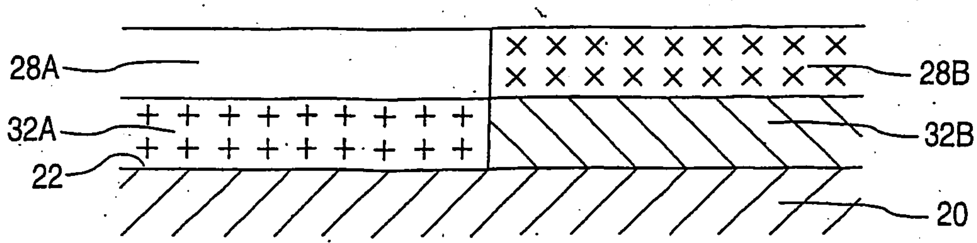

Figure 2C

INTERNATIONAL SEARCH REPORT

PCT/US 02/29340

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61L31/10 A61L31/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 023 879 A (MEDTRONIC INC) 2 August 2000 (2000-08-02) claims; examples ---	1-32
X	US 5 980 972 A (DING NI) 9 November 1999 (1999-11-09) column 3, line 17 - line 51; claims ---	1-32
X	WO 01 45763 A (ADVANCED CARDIOVASCULAR SYSTEM) 28 June 2001 (2001-06-28) claims; examples ---	1-32
X	US 4 977 901 A (OFSTEAD RONALD F) 18 December 1990 (1990-12-18) claims; examples ---	1-32
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 November 2002	25/11/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	ESPINOSA, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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(54) **Process to macrocyclic compounds.**

(57) Compounds of formula I

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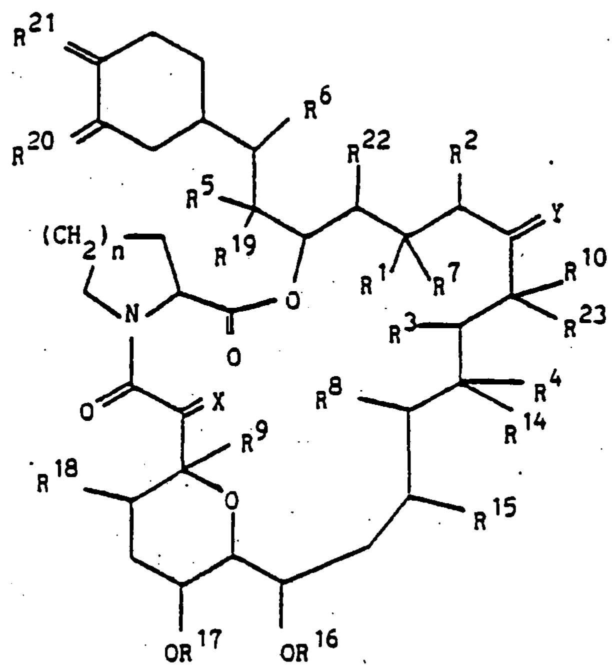

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in which [R^1 and R^2], [R^3 and R^4] and [R^5 and R^6] represent a carbon-carbon bond or two hydrogen atoms;
R^2 additionally represents alkyl;
R^7, R^8 and R^9 represent groups including H or OH,
R^{10} has various significances including alkyl and alkenyl;
X and Y represent groups including O and (H,OH);
R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{22} and R^{23} represent H or alkyl;
R^{20} and R^{21} represent groups including O, (H,OH) and (H,O-alkyl),
n is 1, 2, or 3,
and in addition, Y, R^{10} and R^{23}, together with the carbon atoms to which they are attached, may represent a neterocyclic ring,
(with certain provisos) are described.

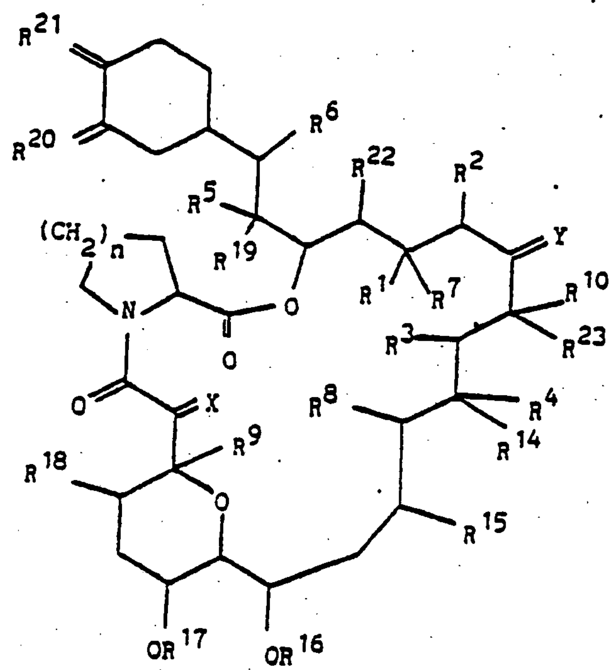
Processes for making the compounds and pharmaceutical formulations containing them, eg for use as immunosuppressive agents, are also described.

PROCESS TO MACROCYCLIC COMPOUNDS

This invention relates to novel compounds, methods for their preparation, their use as medicaments, and compositions containing them.

European Patent Application 0184162 (to Fujisawa Pharmaceuticals Co. Ltd.) discloses a number of macrolides isolated from microorganisms belonging to the genus Streptomyces. The macrolides are numbered FR-900506, FR-900520, FR-900523 and FR-900525, and may be used as starting materials to produce the compounds of the present invention.

According to the invention, we provide compounds of formula I,



I

wherein each vicinal pair of substituents [R^1 and R^2], [R^3 and R^4], [R^5 and R^6] independently

a) represent two vicinal hydrogen atoms, or

b) form a second bond between the vicinal carbon atoms to which they are attached;

in addition to its significance above, R^2 may represent an alkyl group;

R^7 represents H, OH or O-alkyl, or in conjunction with R^1 it may represent =O,

R^8 and R^9 independently represent H or OH;

R^{10} represents H, alkyl, alkyl substituted by one or more hydroxyl groups, alkenyl, alkenyl substituted by one or more hydroxyl groups, or alkyl substituted by =O;

X represents O, (H,OH) (H,H) or $-CH_2O-$;

Y represents O, (H,OH), (H,H), N-$NR^{11}R^{12}$ or N-OR^{13};

R^{11} and R^{12} independently represent H, alkyl, aryl or tosyl;

R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{22} and R^{23} independently represent H or alkyl;

R^{20} and R^{21} independently represent 0, or they may independently represent (R^{20}a,H) and (R^{21}a,H) respectively; R^{20}a and R^{21}a independently represent OH, O-alkyl or $OCH_2OCH_2CH_2OCH_3$,

in addition, R^{20}a and R^{21}a may together represent an oxygen atom in an epoxide ring;

n is 1, 2 or 3;

in addition to their significances above, Y, R^{10} and R^{23}, together with the carbon atoms to which they are attached, may represent a 5- or 6- membered N-, S- or O- containing heterocyclic ring, which may be saturated or unsaturated, and which may be substituted by one or more groups selected from alkyl, hydroxyl, alkyl substituted by one or more hydroxyl groups, O-alkyl, benzyl and $-CH_2Se(C_6H_5)$';

provided that when X and Y both represent O; R^9 represents OH; R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19} and R^{22} each represent methyl; $R^{20}a$ represents OCH_3; R^8 and R^{23} each represent H; $R^{21}a$ represents OH; [R^3 and R^4] and [R^5 and R^6] each represent a carbon-carbon bond, and

a) when n = 1, R^7 represents an OH group and R^1 and R^2 each represent hydrogen, then R^{10} does not represent an allyl group,

b) when n = 2, R^7 represents OH and R^1 and R^2 each represent hydrogen, then R^{10} does not represent methyl, ethyl, propyl or allyl, and

c) when n = 2, R^7 represents hydrogen and [R^1 and R^2] represents a carbon-carbon bond, then R^{10} does not represent an allyl group;

and pharmaceutically acceptable salts thereof.

Pharmaceutically acceptable salts of the compounds of formula I include acid addition salts of any amine groups present.

Preferably when R^2, R^7, R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, $R^{20}a$, $R^{21}a$, R^{22} and R^{23} comprise carbon-containing groups, those groups contain up to 10 carbon atoms, more preferably from 1 to 6, eg methyl or methoxyl.

We prefer each of R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19} and R^{22} to represent methyl.

Alkyl groups which R^2, R^7, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, $R^{20}a$, $R^{21}a$, R^{22} and R^{23} may comprise include straight chain, branched and cyclic groups.

Alkyl groups substituted by =O which R^{10} may represent include ketone and aldehyde groups.

Preferably, R^{10} is allyl (ie prop-2-enyl), propyl, ethyl or methyl.

Preferably, n is 2.

We prefer R^7 to be H or OH.

Preferably, R^1 and R^2 both represent H.

X is preferably O or (H,OH).

We prefer $R^{20}a$ and $R^{21}a$ to (independently) represent OH or OCH_3.

When Y, R^{10} and R^{23} together represent a N-,S- or O- containing heterocyclic ring, we prefer that ring to be five-membered, more preferably a pyrrole or tetrahydrofuran ring.

According to the invention there is further provided a process for the preparation of compounds of formula I, or pharmaceutically acceptable salts thereof. The starting material for a compound of the present invention is preferably one of the macrolides isolated from microorganisms of the genus Streptomyces, which are disclosed in European Patent Application 0184162. One or more processes discussed below may be employed to produce the desired compound of the invention.

Such a process comprises:

(a) producing a compound of formula I, in which one or more of [R^1 and R^2], [R^3 and R^4] or [R^5 and R^6] represent two vicinal hydrogen atoms, by selective reduction of a corresponding group [R^1 and R^2], [R^3 and R^4] or [R^5 and R^6] when it represents a second bond between two vicinal carbon atoms in a corresponding compound,

(b) producing a compound of formula I, which contains one or more hydroxyl groups, by selective reduction of one or more C=O groups in a corresponding compound,

(c) producing a compound of formula I, which contains a

$$\underset{\mathrm{OH}}{\overset{|}{\mathrm{C}}}\!=\!\mathrm{C}\!-\!\mathrm{C}\!=\!\mathrm{O}$$

group, by selective oxidation of a $\underset{\mathrm{OH}}{\mathrm{C}}\!-\!\mathrm{C}\!-\!\mathrm{C}\!=\!\mathrm{O}$ group in a corresponding compound,

(d) producing a compound of formula I, which contains one or more alkoxy groups, by alkylation of one or more hydroxyl groups in a corresponding compound by reaction with a suitable alkylating agent,

(e) producing a compound of formula I, which contains one or more hydroxyl groups, by deprotection of one or more protected hydroxyl groups in a corresponding compound where the protecting group is preferably removable by hydrogenolysis,

(f) producing a compound of formula I, which contains a carbon-carbon double bond, by elimination of HL from a corresponding compound, where L is a leaving group,

(g) producing a compound of formula I, in which Y, R^{23} and R^{10}, together with the carbon atoms to which they are attached, form a tetrahydrofuran ring substituted by a $CH_2Se(C_6H_5)$ group, by reacting a phenylselenyl halide with a corresponding compound in which Y is O and R^{10} is allyl,

(h) producing a compound of formula I, which contains an allylic alcohol, by selective oxidation of an allyl group in a corresponding compound,

(i) producing a compound of formula I, which contains a ketone group, by oxidation of a hydroxyl group in a corresponding compound,

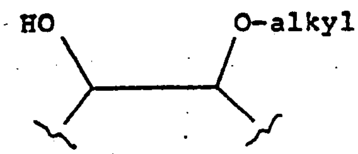
(j) producing a compound of formula I, which contains a vicinal diol, by oxidation of a carbon-carbon double bond in a corresponding compound,

(k) producing a compound of formula I, which contains an aldehyde group, by oxidative cleavage of a vicinal diol in a corresponding compound,

(l) producing a compound of formula I, in which Y, R^{10} and R^{23}, together with the carbon atoms to which they are attached,form a pyrrole ring, by reacting ammonia or an amine with a corresponding compound in which Y is O and R^{10} is $-CH_2CHO$,

(m) producing a compound of formula I, which contains an epoxide group, by cyclization of

an



group in a corresponding compound,

(n) producing a compound of formula I, in which Y represents an oxime group, by reaction of a corresponding compound in which Y is O with an oxygen-substituted amine.

(o) producing a compound of formula I, which contains a $COCH_3$ group, by oxidation of a terminal alkene group in a corresponding compound,

(p) producing a compound of formula I, in which X represents $-CH_2O-$, by reacting a corresponding compound in which X is O with diazomethane, or

(q) producing a compound of formula I, in which Y is a hydrazone or a hydrazone derivative, by reacting a corresponding compound in which Y is O with hydrazine or a substituted hydrazine.

In process (a), reduction may be carried out catalytically using hydrogen. Suitable catalysts include platinum catalysts (eg platinum black, platinum oxides), palladium catalysts (eg palladium oxide, palladium on charcoal), nickel catalysts (eg nickel oxide, Raney nickel), and rhodium catalysts (eg rhodium on alumina). Suitable solvents are those which do not adversely affect the reaction, and include methanol, ethanol, ethyl acetate, dichloromethane and dimethylformamide. The reduction may be carried out at or around room temperature.

Reduction may also be achieved by other means. For example, when the carbon-carbon double bond is conjugated with a ketone.group, the reduction may be effected using an alkyl tin hydride, for example tri n-butyl tin hydride, in the presence of a catalyst, for example tetrakis(triphenylphosphine) palladium (0) . In this case, the reaction is preferably carried out in a solvent which does noy adversely affect the reaction, for example toluene or benzene, and preferably under slightly acidic conditions, for example in the presence of a trace of acetic acid.

In process (b), suitable reagents include sodium borohydride, zinc in acetic acid, sodium triacetoxyborohydride in acetic acid, L-Selectride (Registered Trade Mark) in tetrahydrofuran, or preferably borane/^{t}butylamine complex in a solvent such as methanol or ethanol. The reduction may be conducted at or around room temperature.

In process (c), suitable oxidizing agents include dialkyl sulphoxides (eg dimethyl-sulphoxide, methylethyl-sulphoxide). The oxidation may be carried out in a solvent which does not adversely affect the reaction (eg acetone, dichloromethane, tetrahydrofuran) in the presence of an alkanoic anhydride. We prefer the anhydride to be acetic anhydride, as this may also function as the solvent for the reaction. The reaction may be conducted at or around room temperature.

In process (d), suitable alkylating agents include alkyl tosylates, diazoalkanes and alkyl halides (eg alkyl chlorides, bromides and iodides). Suitable solvents include those which are inert under the reaction conditions. We prefer polar, aprotic solvents such as dimethylformamide, 1,4-dioxan and acetonitrile. When the alkylating agent is an alkyl halide, the reaction is preferably carried out in the presence of a base, eg potassium carbonate, at a temperature of from about 0 to 100 °C.

In process (e), when the hydroxyl protecting group is hydrogenolysable, hydrogenolysis may be carried out in a solvent which is inert to the reaction conditions, eg in an alcoholic solvent such as ethanol or methanol. Hydrogenolysable hydroxyl protecting groups include arylmethyl groups, in particular substitiuted and unsubstituted phenylmethyl groups. The reaction is preferably carried out using hydrogen at a pressure

of from about 1 to 3 atmospheres using a metal catalyst on a support, eg palladium on charcoal. The hydrogenolysis is preferably carried out at a temperature of from about 0 to 50° C.

In process (f), L may be halogen or hydroxy for example.

When the precursor compound contains a

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C-C-C=O
| |
HO H
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group, the elimination of H_2O may be carried out in a solvent which is inert under the reaction conditions (eg toluene) with a trace amount of acid (eg tosic acid), at a temperature from about 50 to 100° C.

In process (g), the reaction may be conducted by reacting a corresponding compound in which Y is O and R^{10} is allyl, with phenylselenylbromide, using methanol as solvent, at a temperature below 0° C, preferably from -20 to -80° C.

In process (h), suitable oxidizing agents include selenium dioxide (when other oxidizable goups are either absent or protected), preferably in the presence of ^{t}butyl hydrogen peroxide. Suitable solvents include dichloromethane, and the reaction is preferably conducted at a temperature of from 0 to 50° C, more preferably 15-25° C.

In process (i), suitable reagents include acidified sodium dichromate and aluminium t-butoxide (the Oppenauer method). Suitable solvents include acetone in each case; but with sodium dichromate we prefer the solvent to be acetic acid; and with aluminium t-butoxide, benzene or toluene may be added as a co-solvent. Sodium dichromate is preferably used at or around room temperature, while aluminium t-butoxide is preferably used at the reflux temperature of the reaction mixture.

In process (j), suitable reagents include osmium tetroxide, potassium permanganate, and iodine in conjunction with silver acetate. Osmium tetroxide is preferably used with a regenerating agent such as hydrogen peroxide, alkaline t-butyl hydroperoxide or N-methylmorpholine-N-oxide, and a solvent which does not adversely affect the reaction, for example diethyl ether or tetrahydrofuran. Potassium permanganate is preferably used in mild conditions, for example alkaline aqueous solution or suspensions. Co-solvents such as t-butanol or acetic acid may also be used.

Iodine-silver acetate under 'wet' conditions yields cis-diols. Preferably, iodine is used in aqueous acetic acid in the presence of silver acetate. Iodine-silver acetate under 'dry' conditions yields trans-diols. Here, the initial reaction is carried out in the absence of water, and final hydrolysis yields the diol (Prevost reaction). In each case, the oxidation is preferably carried out at a temperature from 0 to 100° C, more preferably at or around room temperature.

In process (k), suitable reagents include lead tetraacetate, phenyliodoso acetate, periodic acid or sodium metaperiodate. Suitable solvents for the first two reagents include benzene and glacial acetic acid. The second two reagents are preferably used in aqueous solution. The reaction is preferably carried out at a temperature of from 0 to 100° C, more preferably at or around room temperature.

In process (1) a pyrrole ring in which the nitrogen atom is unsubstituted may be produced by reacting a corresponding compound in which Y is O and R^{10} is $-CH_2CHO$ with ammonia. Pyrrole rings in which the nitrogen atom is substituted may be produced by reacting the precursor compound with a substituted amine, for example 2-aminoethanol or benzylamine. Suitable solvents include those which do not adversely affect the reaction, for example dichloromethane. The reaction is preferably carried out at a temperature of from 0 to 100° C, more preferably at or around room temperature.

In process (m), suitable reagents include boron trifluoride followed by diazomethane. Suitable solvents are those which do not adversely affect the reaction, for example dichloromethane. The reaction is preferably carried out at a temperature of from 0 to 100° C, more preferably at or around room temperature.

In process (n), suitable oxygen-substituted amines include hydroxyl amine and 0-alkyl hydroxyl-amines, for example O-methyl hydroxylamine. Suitable solvents include those which do not adversely affect the reaction, for example ethanol or methanol. The reaction is preferably carried out at a temperature of from 50-200° C, more preferably at the reflux temperature of the solvent.

In process (o), suitable reagents include a palladium (II) halide, for example palladium (II) chloride, in conjunction with a cuprous halide, for example cuprous chloride. Suitable solvents include those that do not adversely affect the reaction, for example dimethyl formamide and water. The reaction is preferably carried out at a temperature of from 0 to 100° C, more preferably at or around room temperature.

In process (p), suitable solvents include those which do not adversely affect the reaction, for example dichloromethane. the reaction is preferably carried out at a temperature of from 0 to 50° C, more preferably

at or around room temperature.

In process (q), suitable reagents include hydrazine and toluene-4-sulphonylhydrazide. Suitable solvents include those which do not adversely affect the reaction conditions, for example methanol or ethanol. The reaction is preferably carried out at a temperature of from 0 to 50° C, more preferably at or around room temperature.

D. Askin et al (Tetrahedron Letts; 1988, 29, 277), S. Mills et al (ibid; 1988, 29, 281) and D Donald et al (ibid; 1988, 29, 4481) have recently disclosed synthetic routes to fragments of macrolide FR-900506 mentioned above. Their approaches may be incorporated into a process for producing the novel compounds of the present invention, in particular when one or more of R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19} or R^{22} is other than methyl.

The processes described above may produce the compound of formula I or a salt thereof. It is also within the scope of this invention to treat any salt so produced to liberate the free compound of formula I, or to convert one salt into another.

The compounds of formula I, and pharmaceutically acceptable salts thereof, are useful because they possess pharmacological activity in animals; in particular they are useful because they possess immunosuppressive activity, eg in the tests set out in Examples A, B and C. Thus the compounds are indicated for use in the treatment or prevention of the resistance by transplantion of organs or tissues, such as kidney, heart, lung, bone marrow, skin, etc, and of autoimmune and proliferative diseases such as rheumatoid arthritis, systemic lupus erythematosus, Hashimoto's thyroiditis, multiple sclerosis, myasthenia gravis, type 1 diabetes, uveitis, psoriasis, etc. Some of the compounds of the invention are also indicated for use as antimicrobial agents, and thus may be used in the treatment of diseases caused by pathogenic microorganisms and the like.

We therefore provide the use of compounds of formula I (and pharmaceutically acceptable salts thereof) as pharmaceuticals.

Further, we provide the use of a compound of formula I (and pharmaceutically acceptable salts thereof) in the manufacture of a medicament for use as an immunosuppressive agent.

For the above-mentioned therapeutic uses the dosage administered will, of course, vary with the compound employed, the mode of administration, the treatment desired (eg topical, parenteral or oral) and the disease indicated. However, in general, satisfactory results are obtained when the compounds are administered at a dosage of from 0.1 to 200mg per kg of animal body weight. For man the indicated total daily dosage is in the range of from 1mg to 1000mg and preferably from 10mg to 500mg, which may be administered, for example twice weekly, or in divided doses from 1 to 6 times a day or in sustained release form. Thus unit dosage forms suitable for administration, eg oesophageally, comprise from 2mg to 500mg, and preferably lmg to 500mg of the compound preferably admixed with a solid or liquid pharmaceutically acceptable diluent, carrier or adjuvant.

According to our invention we also provide a pharmaceutical composition comprising (preferably less than 80%, and more preferably less than 50% by weight) of a compound of formula I, or a pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable adjuvant, diluent or carrier. Examples of suitable adjuvants, diluents or carriers are: for tablets, capsules and dragees; microcyrstalline cellulose, calcium phosphate, diatomaceous earth, a sugar such as lactose, dextrose or mannitol, talc, stearic acid, starch, sodium bicarbonate and/or gelatin; for suppositories, natural or hardened oils or waxes; and for inhalation compositions, coarse lactose. The compound of formula I, or the pharmaceutically acceptable salt thereof, preferably is in a form having a mass median diameter of from 0.01 to 10 microns. The compositions may also contain suitable preserving, stabilising and wetting agents, solubilisers, sweetening and colouring agents and flavourings. The compositions may, if desired, be formulated in sustained release form. We prefer compositions which are designed to be taken oesophageally and to release their contents in the gastrointestinal tract.

The compounds of formula I, and pharmaceutically acceptable salts thereof, have the advantage that they are less toxic, more efficacious, are longer acting, have a broader range of activity, are more potent, produce fewer side effects, are more easily absorbed or have other useful pharmacological properties, than compounds previously used in the therapeutic fields mentioned above.

The compounds of formula I have a number of chiral centres and may exist in a variety of stereoisomers. The invention provides all optical and stereoisomers, as well as racemic mixtures. The isomers may be resolved or separated by conventional techniques.

Example A

Mixed Lymphocyte Reaction (MLR) I

The MLR test was performed in microtitre plates, with each well containing 5 x 10^5 C57BL/6 responder cells (H-2^b), 5 x 10^5 mitomycin C treated (25ug/ml mitomycin C at 37° C for 30 minutes and washed three times with RPMI 1640 medium) BALB/C stimulator cells (H-2^d) in 0.2ml RPMI 1640 medium supplemented with 10% fetal calf serum, 2mM sodium hydrogen carbonate, penicillin (50 unit/ml) and streptomycin (50ug/ml). The cells were incubated at 37° C in a humidified atmosphere of 5% carbon dioxide and 95% of air for 68 hours and pulsed with ^{3}H-thymidine (0.5uCi) 4 hours before the cells were collected. The object compound of this invention was dissolved in ethanol and further diluted in RPMI 1640 medium and added to the cultures to give final concentrations of 0.1ug/ml or less.

Example B

Mixed Lymphoctye Reaction (MLR) II

The MLR test was performed in 96-well microtitre plates with each well containing 3 x 10^5 cells from each of two responding donors in a final volume of 0.2ml RPMI 1640 medium supplemented with 10% human serum, L-glutamine and penicillin/streptomycin. The compound under test was dissolved at 10mg/ml in ethanol and further diluted in RPMI 1640. The cells were incubated at 37° C in a humidified atmosphere of 5% carbon dioxide for 96 hours. ^{3}H-thymidine (0.5uCi) was added for the final 24 hours of the incubation to provide a measure of proliferation.

Example C

Graft versus Host Assay (GVH)

Spleen cells from DA and DAxLewis Fl hybrid rats were prepared at approximately 10^8 cells/ml. 0.1ml of these suspensions were injected into the rear footpads of DAxLewis F1 rats (left and right respectively). Recipient animals were dosed with the compound under test, either orally or subcutaneously, on days 0-4. The assay is terminated on day 7 when the popliteal lymph nodes of the animals are removed and weighed. The increase in weight of the left node relative to the weight of the right is a measure of the GVH response.

The invention is illustrated by the following Examples.

Example 1

17-Allyl-1-hydroxy-12-[2-(3,4-dimethoxycyclohexyl)-1-methylvinyl]-14,23,25-trimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone.

To a stirred solution of the macrolide FR 900506 (200mg) in dichloromethane (30ml) and ether (20ml) was added boron trifluoride diethyl ether complex (10mg) and then a solution of diazomethane (600mg) in ether (30ml) added slowly over 5 minutes with evolution of nitrogen. The products were purified by chromatography on silica, with ether as the eluant, to yield the title compound (55mg), characterised by nmr and mass spectroscopy.

MS: (FAB) 831 (molecular ion)

^{13}C NMR δ : 210.0 (C16); 196.5(C2); 166.9 (C10); 164.6 (C3); 138.5 (C19); 135.6 (C41); 133.9 (C29); 131.5 (C31); 123.3 (C18); 116.5 (C42); 97.6 (C1); 83.1 (C34); 82.6 (C35); 79.0 (C14)

Example 2

17-Allyl-1,14-dihydroxy-12-[2-(3,4-dimethoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11, 28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10, 16-tetraone

To a stirred solution of macrolide FR 900506 (80mg) in dichloromethane (100ml) and ether (50ml) was added 35-70 micron silica (5g) and then diazomethane (300mg) in ether (50ml) was added slowly over 5 minutes with evolution of nitrogen. After stirring for a further 15 minutes the products were purified by chromatography on silica, with ether as eluant, to yield the title compound (15mg), characterised by nmr and mass spectroscopy.
MS: (FAB) 840.8 (MI + Na)
^{13}C NMR δ : 212.8 (C16); 196.2 (C2); 169.0 (C.10); 164.7 (C3); 139.0 (C19); 135.6 (C41); 132.4 (C29); 129.7 (C31); 122.4 (C18); 116.7 (C42); 97.0 (C1); 83.2 (C34); 82.6 (C35)

Example 3

1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-18-[(phenylseleno)methyl]-16,26,28-trimethoxy-13,22,24,30-tetramethyl-11,17,31-trioxa-4-azatetracyclo[25.3.1.$0^{4,9}$.$0^{16,20}$]hentriacont-21-ene-2,3,10-trione

To a solution of the macrolide FR 900506 (62mg, 7.7x10^{-5}moles) in dry methanol (12ml) at -78° C under nitrogen was added 2,6-dimethylpyridine (9.9mg, 9.24x10^{-5}moles). To this was then added a solution of 0.46mg of phenylselenylbromide in acetonitrile (0.47ml of a solution of 0.46mg of phenylselenylbromide in 1ml of acetonitrile), followed after 20 minutes by 0.53ml of the same solution. The reaction mixture was then evaporated at low temperature in vacuo and the residue was chromatographed on silica eluting with dichloromethane/ethyl acetate (2:1) to give the product as a mixture of diastereoisomers (65.5mg) which could be further separated by HPLC.
MS: (FAB) 1013 (MI + Na).
^{13}C NMR δ : 133.2, 129.2, 127.4 (Ar); 111.6 (C10); 97.5 (C1); 78.0 (C14); 76.7 (C41); 55.8 (C9); 50.3, 49.5 (C10 rotamers); 49.8 (C17); 41.2 (C15); 31.2 (C42); 29.7 (C40).

Example 4

17-Allyl-1-hydroxy-12-[2-(3,4-dimethoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacosa-14,18-diene-2,3,10,16-tetraone

A stirred solution of 17-Allyl-1,14-dihydroxy-12-[2-(3,4-dimethoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone (10mg, prepared by methylation of macrolide FR 900506), in toluene (5ml) containing a trace of tosic acid was heated on a steam bath for 5 minutes. Removal of solvent in vacuo and chromatography on silica eluting with ethyl acetate gave the title compound as an oil (8mg).
MS: (FAB) 822.8 (MI + Na) 800.9 (MI + H)
^{13}C NMR δ : 200.4 (C16); 192.2 (C2); 169.2 (C10); 165.0 (C3); 148.2 (C14); 138.3 (C39); 135.4 (C41); 131.4, 130.0, 127.6 (C15, C29, C31); 124.1 (C18); 116.5 (C42); 97.9 (C1); 83.3 (C34); 83.0 (C35); 79.8 (C12).

Example 5

17-Allyl-1,2,14-trihydroxy-12-[2-(4-hydroxy-3-methoxy cyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

To a solution of the macrolide FR 900506 (100mg) in methanol (5ml) was added a solution of Borane ^{t}Butylamine complex (3.7mg) in methanol (1ml) and the solution was stirred for 12 hours at room temperature. The solution was evaporated and chromatographed on silica using ethyl acetate as eluent to give the title compound (32mg) as a mixture of diastereoisomers.

MS: (FAB) 829 (MI+Na)
^{13}C NMR (showing a mixture of rotamers) δ : 212.0, 213.4 (C16); 171, 172.8 (C10); 170.4, 169.8 (C3); 140, 140.5 (C19); 135.5, 135.6 (C41); 132.4, 132.6 (C29); 29,130 (C31); 122.5 (C18); 116.5 (C42); 99.2, 97.5 (C1).

Example 6

17-Allyl-1,2,14,16-tetrahydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21, 27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1^{4,9}] octacos-18-ene-3,10-dione

To a solution of the macrolide FR 900506 (40mg) in diethyl ether (5ml) was added excess borane ammonia complex (100mg) and the solution was stirred at room temperature for 1 hour. Dilute hydrochloric acid was added and the organic phase was separated and chromatographed on silica using ethyl acetate as eluant to give the title compound (25mg) as a white solid.
MS: (FAB) 830.8 (MI+Na)
^{13}C NMR δ : 174.3 (C10); 171.7 (C3): 116.1 (C42).
Mp 130-150° C.

Example 7

17-Propyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxy cyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}] octacosane-2,3,10,16-tetraone

To a solution of the macrolide FR 900506 (100mg) in methanol was added Pd-on-carbon (20mg) and the mixture was stirred in an atmosphere of hydrogen for 20 hours. Filtration of the reaction mixture, evaporation of the solvent in vacuo and HPLC of the resulting mixture on silica gave the title product (35mg).

Example 8

17-Propyl-1,14-dih droxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatric clo[22.3.1.0^{4,9}]octacos 18-ene-2,3,10,16-tetraone

To a solution of the macrolide FR 900506 (100mg) in methanol was added Pd on Carbon (20mg) and the mixture was stirred in an atmosphere of hydrogen for 20 hours. Filtration of the reaction mixture, evaporation of the solvent in vacuo and HPLC of the resulting mixture on silica gave the title product (30mg).

Example 9

17-Propyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacosane-2,3,10,16-tetraone

To a solution of the macrolide FR 900506 (100mg) in methanol was added Pd on Carbon (20mg) and the mixture was stirred in an atmosphere of hydrogen for 20 hours. Filtration of the reaction mixture, evaporation of the solvent in vacuo and HPLC of the resulting mixture on silica gave the title product (15mg).

Example 10

17-Propyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone

To a solution of the macrolide FR 900506 (800mg) in ethanol (20ml) was added Pd-on-carbon (10mg) and the mixture was stirred in an atmosphere of hydrogen for 30 minutes. Filtration of the reaction mixture, evaporation of the solvent in vacuo and chromatography on silica eluting with ether/methanol (20:1) yielded the title compound as an oil (750mg).
^{13}C NMR δ : 33.32 (C40); 20.43 (C41); 14.11 (C42)

Example 11

17-Propyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacosa-14,18-diene-2,3,10,16-tetraone

A stirred solution of 17-propyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone (800mg prepared by the method of example 10), in toluene (20ml) containing 50mg of tosic acid was heated on a steam bath for 30 minutes. Removal of solvent in vacuo and chromatogaraphy on silica eluting with ether gave the title compound as an oil (600mg).
MS : (FAB) 811 (molecular ion + Na)
^{13}C NMR δ : 34.64 (C40); 20.54 (C41); 14.08 (C42); 201.21 (major), 199.76 (minor) (C16); 147.93 (major), 146.25 (minor).

Example 12

17-Propyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetra methyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone

0.75ml of tributyl tin hydride, 0.06ml of water and 150mg of $(Ph_3P)_4Pd$ were added in three portions over a period of 1 hour to a stirred solution of the title compound of example 11 (600mg) in tetrahydrofuran (20ml) at room temperature. Water was then added, and the mixture extracted into ether. Removal of solvent in vacuo and chromatography on silica eluting with ether gave the title compound as an oil (400mg).
MS : (FAB) 813 (MI + Na)
^{13}C NMR δ : 212.3 (C16); 196.4 (C2); 169.4 (C10); 165.1 (C3); 138.1 (C19); 131.7 (C31); 124.3 (C18); 97.4 (C1); 84.1 (C34); 82.4 (C12).

Example 13

17-Allyl-1,14,20-trihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone and 17-(1-Hydroxyprop-2-enyl)-1,14,20-trihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo [22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone

To a stirred solution of the macrolide FR 900506 (140mg) in dichloromethane (17.5ml) at room temperature was added SeO_2 (700mg), followed by tertiary-butyl hydrogen peroxide (1.05ml of a 70% aqueous solution). The reaction mixture was left to stir for 60 hours, after which it was extracted with ethyl acetate. The organic phase was washed with water followed by brine, dried ($MgSO_4$) and concentrated in vacuo. Flash chromatography on silica eluting with ether/methanol (20:1) yielded the title compounds separately as oils (19mg and 15mg respectively).
^{13}C NMR[$CDCl_3$] δ :
(first compound) 141.4(C19); 123.5(C18); 135.4(C41); 117.0(C42); 131.6(C29); 129.1(C13); 211.4(C16); 195.4(C2); 170.4(C10); 166.7(C3); 98.2(C1) and 84.1(C34)ppm.

(second compound) 142.3(C19); 120.7(C18); 137.5(C41); 115.9(C42); 132.3(C29); 129.0(C31); 210.7(C16); 195.8(C2); 170.5(C10); 167.2(C3); 98.2(C1) and 84.1(C34) ppm
MS: (FAB) (first compound) 904 (MI+Rb), 842 (MI+Na);
(second compound) 920 (MI+Rb), 859 (MI+Na);

Example 14

17-Allyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacosa-4,18-diene-2,3,10,16-tetraone

A stirred solution of the macrolide FR 900506 (200mg) in dry toluene (10ml) containing 5mg of tosic acid was heated on a steam bath for 30 minutes. Removal of solvent in vacuo and chromatography on silica eluting with ether gave the title compound as an oil (160mg).
MS: (FAB) 808 (MI+Na); 786 (MI+H).
^{13}C NMR δ : 200.4 (C16); 196.0 (C2); 169.3 (C10); 65.0 (C3); 148.0 (C14); 138.3 (C39); 135.5 (C41); 23.4 (C18); 116.6 (C42); 98.0 (C1); 84.2 (C34); 9.8 (C12).

Example 15

17-Allyl-1,2-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione

To a stirred solution of the title compound of example 14 (60mg) in glacial acetic acid (5ml) was added powdered zinc (1g). Stirring was continued for 1 hour when the reaction was complete. The reaction mixture was then extracted with ethyl acetate, washed with saturated $NaHCO_3$ solution followed by brine, dried ($MgSO_4$) and concentrated in vacuo. Chromatography on silica eluting with ether/methanol (15:1 then 10:1) gave the title compound as an oil (30mg). ^{13}C NMR[$CDCl_3$] δ : 99.1(C1); 67.9(C2); 171.2 and 171.7(C10 and C3); 44.6(C5); 83.32(C12); 84.0(C34); 76.6(C23); 71.7(C24); 73.3 and 73.9(C25 and C35); 52.9(C9); 52.7-(C17) and 49.5(C20) ppm.
MS: (FAB) 874 (MI+Rb); 813 (MI+Na).

Example 16

17-Allyl-1,16-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacosa-14, 18-diene-2,3,10-trione

The title compound from example 14 (50mg) was dissolved in tetrahydrofuran (3ml) and t-butanol (0.05ml). The resulting solution was added dropwise to a stirred solution of L-Selectride (Registered Trade Mark) (0.3ml of a 1M solution in tetrahydrofuran) under a nitrogen atmosphere at -78°C. Stirring was continued for 40 minutes, after which saturated ammonium chloride solution (5ml) was added and the mixture extracted with ethyl acetate. After filtration of the organic phase, and removal of solvent in vacuo chromatography on silica eluting with ether/methanol (15:1) yielded the title compound as an oil (10mg).
^{13}C NMR[$CDCl_3$] δ : 197.0(C2); 169.1(C10); 165.3(C3); 96.4(Cl) and 84.2(C34) ppm.
MS: (FAB) 872 (MI+Rb); 810 (MI+Na).

Example 17

17-Allyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone

To a stirred solution of 17-Allyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-14,18-diene-2,3,10,16-tetraone (as prepared in Example 4) (100mg) in toluene (5ml) and acetic acid (0.01 ml) was added tetrakis-(triphenylphosphine) palladium(0)(0.01g). After 5 minutes tri n-butyltin hydride (0.04g) was added and the reaction mixture was stirred at room temperature for 2 hours. Water was added and the reaction mixture was extracted with ether. The ether extracts were dried (magnesium sulphate), filtered and evaporated to an oil in vacuo. Chromatography on silica eluting with ether gave the title compound as a low melting solid (70mg).
MS: (FAB) 810.7 (MI + Na), 788.7 (MI + H).
^{13}C NMR δ : 211.3 (C16); 196.3 (C2); 169.2 (C10); 164.9 (C3); 138.4 (C19); 135.5 (C41); 131.6 (C29); 130.9 (C31); 123.3 (C18); 116.3 (C42); 97.2 (C1); 84.0 (C34); 82.2 (C12).

Example 18

17-Propyl-1-hydroxy-12-[2-(3-methoxy-4-oxocyclohexyl)-1 -methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone.

To a stirred solution of the title compound from Example 12 (25mg) in acetic acid (5ml) was added potassium dichromate (25mg), and stirring was continued overnight. The solution was then evaporated to dryness. Chromatography on silica using ether as eluant gave the title compound (15mg).
MS: (FAB) 810 (MI + Na); 788 (MI + H).
^{13}C NMR δ : 212.2 (C35); 208.7 (C16); 196.3 (C2); 169.4 (C10; 165.2 (C3); 138.2 (C19); 132.5 (C29) 129.2 -124.2 (C31-C42); 97.3 (C1); 83.0 (C34); 82.0 (C12)

Example 19

17-(2,3-Dihydroxypropyl)-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27,-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone.

A solution of macrolide 900506 (70mg), N-methylmorpholine-N-oxide (NMO) (70mg), osmium tetroxide (4mg) and water (0.1ml) in tetrahydrofuran (5ml) was stirred at room temperature for 2 hours, and then treated with powdered sodium metabisulphite (100mg) and Florisil (Registered Trade Mark). The mixture was diluted with ethyl acetate, filtered through celite, then washed with saturated $NaHCO_3$ solution, followed by brine. The solution was dried ($MgSO_4$) and concentrated in vacuo to yield the crude title compound.
MS: (FAB) 921 (MI + Rb), 861 (MI + Na).

Example 20

17-Ethanayl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2t3,10,16,-tetraone.

The crude title compound from Example 19 was dissolved in benzene (5ml) and treated with lead tetraacetate (100mg) for 2-3 minutes at room temperature. The solution was then diluted with ethyl acetate, washed with saturated $NaHCO_3$ solution followed by brine, dried ($MgSO_4$) and concentrated in vacuo to yield the crude product.
MS: (FAB) 889 (MI + Rb), 829 (MI + Na)

Example 21

1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-26,28-dimethoxy-13,22,24,30-

tetramethyl-11,31-dioxa-4,17-diazatetracyclo[25.3.1.$0^{4,9}$.$0^{16,20}$]hentriaconta-16(20),18,21-triene-2,3,10-trione.

The crude title compound from Example 20 was dissolved in dichloromethane and treated with 0.88M NH_3 (aq) (0.2ml). After stirring for 5 minutes at room temperature the solution was diluted with ethyl acetate, washed with saturated $NaHCO_3$ solution, dried ($MgSO_4$) and concentrated in vacuo. Chromatography on silica yielded the title compound (18mg).
MS: (FAB) 872 (MI + Rb), 787 (MI).
^{13}C NMR δ : 196.44 (C2); 169.67 (C10); 165.44 (C3); 97.53 (C1).

Example 22

1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-26,28-dimethoxy-17-(2-hydroxyethyl)-13,22,24,30-tetramethyl-11,31-dioxa-4,17-diazatetracyclo[25.3.1.$0^{4,9}$.$0^{16,20}$]hentriaconta-16(20),18,21-triene-2,3,10-trione.

Following the method of Example 21, the title compound (25mg) was prepared by treating the title compound of Example 20 with 2-aminoethanol (0.2ml).
MS: (FAB) 915 (MI + Rb), 831 (M + H).
^{13}C NMR δ : 196.50 (C2); 169.32 (C10); 165.50 (C3); 97.15 (C1).

Example 23

1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-26,28-dimethoxy-13,22,24,30-tetramethyl-17-phenylmethyl-11,31-dioxa-4,17-diazatetracyclo[25.3.1.$0^{4,9}$.$0^{16,20}$]hentriaconta-16(20),18,21-triene-2,3,10-trione.

Following the method of Example 21, the title compound (30mg) was prepared by treating the title compound of Example 20 with benzylamine (0.1ml).
MS: (FAB) 960 (MI + Rb), 876 (MI).
^{13}C NMR δ : 196.44 (C2); 169.67 (C10); 165.44 (C3); 97.53 (C1).

Example 24

17-Allyl-1-hydroxy-12-[2-(3,4-epoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetrameth 1-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacosa-14,18-diene-2,3,10,16-tetraone

To a solution of the title compound of Example 14 (823mg, 1.05mmole) in dry dichloromethane (50ml) was added boron trifluoride diethyl etherate (1 drop) followed by portionwise addition of a dried solution of diazomethane in diethyl ether until no starting material remained. Sodium carbonate was then added, and the resulting mixture stirred for 30 minutes at room temperature. The reaction mixture was then filtered, concentrated in vacuo, and chromatographed on silica eluting with 40-60° petroleum ether/ethyl acetate [3:1] to give the title compound as an oil (45mg).
^{13}C NMR δ : 51.3 (C34/C35)
MS: (FAB) 838.64 (MI + Rb), 776.85 (MI + Na).

Example 25

17-Allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3- methoxycyclohexyl-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone C16 oxime.

A mixture of macrolide 900506 (40mg), hydroxylamine hydrochloride (40mg) and pyridine (20mg) in ethanol (5ml) was heated under reflux for 3 hours. The solution was poured into dilute hydrochloric acid and extracted into dichloromethane. The organic phase was separated and chromatographed on silica, eluting with ethyl acetate to yield the title compound as a colourless solid (25mg).
A 1:1 mixture of syn and anti oximes was present.
^{13}C NMR δ : 196.8 (C2); 169.0 (C10); 165.2 (C3); 162.0 (C16); 138.7 (C19); 135.9 (C41); 132.3 (C29); 129.0 (C31); 125.2 (C18); 116.0 (C42); 97.6 (C1); 84.3 (C34).
MS: (FAB) 834 (MI).

Example 26

17-Allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone C16 oxime O-methyl ether.

A mixture of macrolide 900506 (100mg), O-methyl hydroxylamine hydrochloride (40mg) and pyridine (50mg) in ethanol (5ml) was heated under reflux for 3 hours. The solvent was evaporated and the product chromatographed on silica eluting with ethyl acetate to give the product as a colourless solid (50mg).
A 1:1 mixture of syn and anti oximes was present.
^{13}C NMR δ : 196.4 (C2); 169.1 (C10); 165.2 (C3); 160.1 (C16); 138.2 (C19); 135.8 (C41); 132.6 (C29); 128 (C31); 125 (C18); 116.2 (C42); 97.0 (C1); 84.2 (C34); 61.7 (=NOCH$_3$); 56.2 (C17).
MS: (FAB) 833 (MI+H)

Example 27

17-Ally-1,14-dihydroxy-12-[2-(4-(2′,5′-dioxahexyloxy)-3-methoxycyclohexyl-1-methylvinyl-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2-3,10,16-tetraone.

To a solution of macrolide 900506 (100mg) in dichloromethane (2ml) was added 2-methoxyethoxymethyl (MEM) chloride (155mg) and N,N-diisopropylethylamine (160mg). After stirring for 90 minutes at room temperature, volatiles were removed in vacuo and the reaction mixture was purified by chromatography on silica eluting with 40-60° petroleum ether/acetone [3:1] to give the title compound as an oil (72mg).
MS: (FAB) 915 (MI+Na)
^{13}C NMR δ : 95 (Cl′MEM); 71.7 and 66.7 (C3′ and C4′ MEM); 58.97 (C6′MEM); 30.7 (C36); 79.4 (C35); 82.6 (C34).

Example 28

17-Propyl-1-hydroxy-12-[2-(3,4-dihydroxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone.

To a solution of 17-Allyl-1,14-dihydroxy-12-[2-(3,4-dihydroxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2,3,10,16-tetraone (100mg) in toluene (20ml) was added p-toluenesulphonic acid (5mg) and the resulting solution was warmed for 90 minutes on a steam bath. Evaporation of volatiles in vacuo and chromatography of the residue on silica eluting with ethyl acetate gave an oil which was dissolved in methanol (5ml). To this was then added 10% palladium-on-carbon (20mg) and the mixture was stirred in an atmosphere of hydrogen for 1 hour at room temperature. The reaction mixture was then filtered through celite, concentrated in vacuo and purified by chromatography on silica eluting with ethyl acetate to give the title compound as an oil (40mg).
MS:(FAB) 799 (M+Na) 861 (M+Rb)

Example 29

1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-17-(2-oxopropyl)-11,28,-dioxa-4-azatricyclo[22.3.1.0^{4,9}] octacos-18-ene-2,3,10,16-tetraone.

A solution of macrolide 900506 (250mg) in dimethylformamide (2ml) was added to a stirred mixture of cuprous chloride (150mg) and palladium (2) chloride (50mg) in dimethylformamide (6ml) and water (1.2ml) at room temperature. A slow stream of air was passed through the reaction mixture which was stirred at room temperature for 1.5 hours. The reaction mixture was then diluted with diethyl ether (200ml), washed with dilute aqueous hydrochloric acid (1Mx2) and brine, dried (magnesium sulphate), filtered and concentrated to an oil in vacuo. Chromatography on silica eluting with hexane/acetone [2:1] gave the title compound as a foam (158mg).
MS (FAB) 821 (MI+H), 843 (MI+Na), 905 (MI+Rb).
^{13}C NMR δ : (major isomer) 97.11 (Cl); 196.02 (C2); 164.60 (C.3); 168.74 (C10); 213.04 (C16); 120.83 (C18); 138.51 (C19); 132.71 (C29); 129.07 (C31); 29.64 (C42); 207.74 (C41).
(minor isomer) 98.29 (C1); 193.33 (C2); 165.75 (C3); 168.67 (C10); 212.90 (C16); 120.47 (C18); 140.29 (C19); 132.17 (C29); 129.29 (C31); 207.86 (C41).

Example 30

17-Allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-aza-spiro[tricyclo [22.3.1.0.^{4,9}]octacos-18-ene-2,2 -oxirane]-3,10,16-trione.

A solution of diazomethane (excess) in dry ether was added to a solution of macrolide 900506 (50mg) in dichloromethane (5ml). The solution was stirred for 2 hours, then chromatographed on silica using ethyl acetate as eluant. The title compound was obtained as a colourless solid.
MS: (FAB) 818 (MI).
^{13}C NMR δ : 212 (C16); 170.4 (C10); 165.7 (C3); 139.0 (C19); 135.4 (C41); 132.4 (C29); 129.4 (C31); 132.0 (C18); 116.8 (C42); 96.7 (C1); 84.2 (C34); 61.6 (C2) 50.7 (C2a)

Example 31

17-Ethanalyl-1,2,14-trihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl-1-methylvinyl]-23,25-dimethoxy-13,19 21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-3,10,16-trione.

A sample of the crude product from Example 20 (15mg) was dissolved in acetic acid (3ml).

Zinc dust (0.5g) was then added and the mixture was stirred at room temperature for 30 minutes. After aqueous work up and column chromatography on silica the title compound was isolated as an oil (10mg).
^{13}C NMR δ : (1:1 mixture of rotamers) 99.08, 97.75 (C1); 212.81, 209.84 (C16); 200.61, 200.27 (C41); 172.40, 171.25, 170.41, 169.84 (C10, C3); 141.28, 140.96 (C29); 133.06, 132.50 (C29); 130.32, 128.69 (C31); 121.07, 120.47 (C18).
MS: (FAB) 892 (MI+Rb), 831 (MI+Na).

Example 32

1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-26,28-dimethoxy-18-[(phenylseleno)-methyl]-13,22,24,30-tetramethyl-11,17,31-trioxa-4-azatetracyclo[25.3.1.0.^{4,9}0.^{16,20}]hentriaconta-16(20),21-diene-2,3,10-trione.

To a cold (-78°C) solution of macrolide 900506 (198.5mg) and 2,6-dimethylpyridine (29mg) in dry methanol (8ml) was added a solution of phenylselenyl bromide (127.3mg) in dry acetonitrile (2.2ml) under

nitrogen. Solvents were then removed in vacuo at low temperature and the residual oil was purified by column chromatography on silica eluting with dichloromethane/ethyl acetate [2:1] to yield the title compound as an oil (37mg).

MS: (FAB) 959 (MI + H)

^{13}C NMR δ : 29.72 (C19); 75.76 (C18); 106.7 (C20); 153.24 (C16).

Example 33

Benzenesulphonic acid, 4′-methyl-[17-Allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0^{4,9}]octacos-18-ene-2-3, 10-trione-16-ylidene]hydrazide.

To a stirred solution of macrolide 900506 (23mg) in ethanol (3ml) was added toluene-4-sulphonyl-hydrazide (5.33mg) and toluene-4-sulphonic acid (5.45mg). The reaction mixture was stirred overnight at room temperature. Solvents were evaporated in vacuo and the residue was purified by column chromatography on silica, eluting with diethyl ether, to yield the title compound (5mg) as an oil.

MS: (FAB) 954 (MI-OH), 972 (MI + H), 994 (MI + Na)

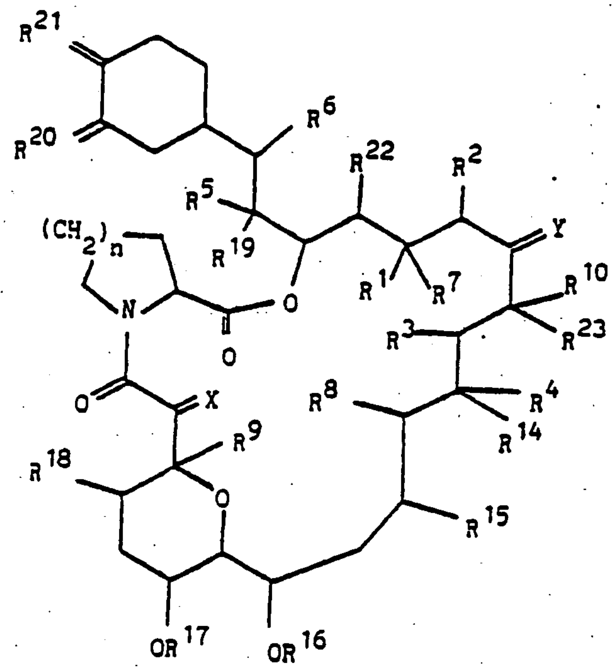
Example 34

A selection of compounds were tested according to Example B. The concentration of compound required to inhibit the proliferation of lymphocytes by 50% was measured, and the results were as follows:

Example No. of product compound	IC_{50} (M)
1	< $1x10^{-6}$
2	< $1x10^{-6}$
5	< $1x10^{-6}$
6	< $1x10^{-6}$
12	< $1x10^{-6}$
15	< $1x10^{-6}$
17	< $1x10^{-6}$
18	< $1x10^{-6}$
22	< $1x10^{-6}$
25	< $1x10^{-6}$
27	< $1x10^{-6}$
30	< $1x10^{-6}$
32	< $1x10^{-6}$

Claims

1. A process for the production of a compound of formula I,



I

wherein each vicinal pair of substituents [R^1 and R^2], [R^3 and R^4], [R^5 and R^6] independently
a) represent two vicinal hydrogen atoms, or
b) form a second bond between the vicinal carbon atoms to which they are attached;
in addition to its significance above, R^2 may represent an alkyl group;
R^7 represents H, OH or O-alkyl, or in conjunction with R^1 it may represent =O,
R^8 and R^9 independently represent H or OH;
R^{10} represents H, alkyl, alkyl substituted by one or more hydroxyl groups, alkenyl, alkenyl substituted by one or more hydroxyl groups, or alkyl substituted by =O;
X represents O, (H,OH), (H,H) or -CH_2O-;
Y represents O, (H,OH), (H,H), N-$NR^{11}R^{12}$ or N-OR^{13};
R^{11} and R^{12} independently represent H, alkyl, aryl or tosyl;
R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19},R^{22} and R^{23} independently represent H or alkyl;
R^{20} and R^{21} independently represent O, or they may independently represent (R^{20}a,H) and (R^{21}a,H) respectively; R^{20}a and R^{21}a independently represent OH, O-alkyl or $OCH_2OCH_2CH_2OCH_3$,
in addition, R^{20}a and R^{21}a may together represent an oxygen atom in an epoxide ring;
n is 1, 2 or 3;
in addition to their significances above, Y, R^{10} and R^{23}, together with the carbon atoms to which they are attached, may represent a 5- or 6- membered N-, S- or O- containing heterocyclic ring, which may be saturated or unsaturated, and which may be substituted by one or more groups selected from alkyl, hydroxyl, alkyl substituted by one or more hydroxyl groups, O-alkyl, benzyl and -$CH_2Se(C_6H_5)$;
provided that when X and Y both represent O; R^9 represents OH; R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19} and R^{22} each represent methyl; R^{20}a represents OCH_3; R^8 and R^{23} each represent H; R^{21}a represents OH; [R^3 and R^4] and [R^5 and R^6] each represent a carbon-carbon bond, and
a) when n = 1, R^7 represents an OH group and R^1 and R^2 each represent hydrogen, then R^{10} does not represent an allyl group,
b) when n = 2, R^7 represents OH and R^1 and R^2 each represent hydrogen, then R^{10} does not represent methyl, ethyl, propyl or allyl, and
c) when n = 2, R^7 represents hydrogen and [R^1 and R^2] represents a carbon-carbon bond, then R^{10} does not represent an allyl group;

and pharmaceutically acceptable salts thereof, which comprises:

(a) producing a compound of formula I, in which one or more of [R^1 and R^2], [R^3 and R^4] or [R^5 and R^6] represent two vicinal hydrogen atoms, by selective reduction of a corresponding group [R^1 and R^2], [R^3 and R^4] or [R^5 and R^6] when it represents a second bond between two vicinal carbon atoms in a corresponding compound,

(b) producing a compound of formula I, which contains one or more hydroxyl groups, by selective reduction of one or more C=O groups in a corresponding compound,

(c) producing a compound of formula I, which contains a $\underset{\mathrm{OH}}{\underset{|}{\mathrm{C}}}=\mathrm{C}-\mathrm{C}=\mathrm{O}$ group, by selective oxidation of a $\underset{\mathrm{OH}}{\underset{|}{\mathrm{C}}}-\mathrm{C}-\mathrm{C}=\mathrm{O}$ group in a corresponding compound,

(d) producing a compound of formula I, which contains one or more alkoxy groups, by alkylation of one or more hydroxyl groups in a corresponding compound by reaction with a suitable alkylating agent,

(e) producing a compound of formula I, which contains one or more hydroxyl groups, by deprotection of one or more protected hydroxyl groups in a corresponding compound where the protecting group is preferably removable by hydrogenolysis,

(f) producing a compound of formula I, which contains a carbon-carbon double bond, by elimination of HL from a corresponding compound, where L is a leaving group,

(g) producing a compound of formula I, in which Y, R^{23} and R^{10}, together with the carbon atoms to which they are attached, form a tetrahydrofuran ring substituted by a $CH_2Se(C_6H_5)$ group by reacting a phenylselenyl halide with a corresponding compound in which Y is O and R^{10} is allyl,

(h) producing a compound of formula I, which contains an allylic alcohol, by selective oxidation of an allyl group in a corresponding compound,

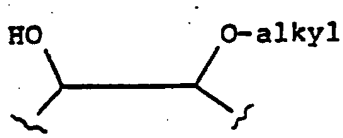
(i) producing a compound of formula I, which contains a ketone group, by oxidation of a hydroxyl group in a corresponding compound,

(j) producing a compound of formula I, which contains a vicinal diol, by oxidation of a carbon-carbon double bond in a corresponding compound,

(k) producing a compound of formula I, which contains an aldehyde group, by oxidative cleavage of a vicinal diol in a corresponding compound,

(l) producing a compound of formula I, in which Y, R^{10} and R^{23}, together with the carbon atoms to which they are attached, form a pyrrole ring, by reacting ammonia or an amine with a corresponding compound in which Y is O and R^{10} is $-CH_2CHO$,

(m) producing a compound of formula I, which contains an epoxide group, by cyclization of an



group in a corresponding compound,

(n) producing a compound of formula I, in which Y represents an oxime group, by reaction of a corresponding compound in which Y is O with an oxygen-substituted amine.

(o) producing a compound of formula I, which contains a $COCH_3$ group, by oxidation of a terminal alkene group in a corresponding compound,

(p) producing a compound of formula I, in which X represents $-CH_2O-$, by reacting a corresponding compound in which X is O with diazomethane, or

(q) producing a compound of formula I, in which Y is a hydrazone or a hydrazone derivative, by reacting a corresponding compound in which Y is O with hydrazine or a substituted hydrazine.

2. A process according to claim 1, wherein R^{10} represents allyl, propyl, ethyl or methyl.

3. A process according to any one of the preceding claims, wherein at least one of R^2,R^{11},R^{12},R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, and R^{22} represents a methyl group.

4. A process according to any one of the preceding claims, wherein at least one of R^{20}a and R^{21}a represent OH or OCH_3.

5. A process according to any one of the preceding claims, wherein n is 2.

6. A process according to any one of the preceding claims, wherein R^7 represents H or OH.

7. A process according to claim 1 wherein the compound of formula I is

17-Allyl-1-hydroxy-12-[2-(3,4-dimethoxycyclohexyl)-1-methylvinyl]-14,23,25-trimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,

17-Allyl-1,14-dihydroxy-12-[2-(3,4-dimethoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,

1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-18-[(phenylseleno)methyl]-16,26,28-trimethoxy-13,22,24,30-tetramethyl-11,17,31-trioxa-4-azatetracyclo[25.3.1.$0^{4,9}$.$0^{16,20}$]hentriacont-21-ene-2,3,10-trione,

17-Allyl-1-hydroxy-12-[2-(3,4-dimethoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacosa-14,18-diene-2,3,10,16-tetraone,

17-Allyl-1,2,14-trihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione,

17-Allyl-1,2,14,16-tetrahydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,7-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$] octacos-18-ene-3,10-dione,

17-Propyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacosane-2,3,10,16-tetraone,

17-Propyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,

17-Propyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacosane-2,3,10,16-tetraone,

17-Propyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacosa-14,18-diene-2,3,10,16-tetraone,

17-Propyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,

17-Allyl-1,14,20-trihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,

17-(1-Hydroxyprop-2-enyl)-1,14,20-trihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,

17-Allyl-1,2-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione,

17-Allyl-1,16-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacosa-14,18-diene-2,3,10-trione,

17-Allyl-1-hydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,7-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$] octacos-18-ene-2,3,10,16-tetraone,

17-Propyl-1-hydroxy-12-[2-(3-methoxy-4-oxocyclohexyl)-1--methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,

17-(2,3-Dihydroxypropyl)-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy13,19,21,27,-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,

17-Ethanalyl-1,14-dihydroxy-12-[2-(4-hydroxy-3- methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16,-tetraone,

1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-26,28-dimethoxy-13,22,24,30-tetramethyl-11,31-dioxa-4,17-diazatetracyclo[25.3.l.$0^{4,9}$.$0^{16,20}$]hentriaconta-16(20),18,21-triene-2,3,10-trione,

1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-26,28-dimethoxy-17-(2-hydroxyethyl)-13,22,24,30-tetramethyl-11,31-dioxa-4,17-diazatetracyclo[25.3.1.0.^{4,9}.$0^{16,20}$]hentriaconta-16(20),18,21-triene-2,3,10-trione,

1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-26,28-dimethoxy-13,22,24,30-tetramethyl-17-phenylmethyl-11,31-dioxa-4,17-diazatetracyclo[25.3.1.$0^{4,9}$ $0^{16,20}$ hentriaconta-16(20),18,21-triene-2,3,10-trione,

17-Allyl-1-hydroxy-12-[2-(3,4-epoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacosa-14,18-diene-2,3,10,16-tetraone,

17-Allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$] octacos-18-ene-2,3,10,16-tetraone C16 oxime,

17-Allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone C16 oxime O-methyl ether,

17-Ally-1,14-dihydroxy-12-[2-(4-(2′,5′-dioxahexyloxy)-3-methoxycyclohexyl-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,

17-Propyl-1-hydroxy-12-[2-(3,4-dihydroxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-

tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,
1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-17-(2-oxopropyl)-11,28,-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone,
17-Allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-aza-spiro[tricyclo[22.3.1.0.^{4,9}]octacos-18-ene-2,2′-oxirane,-3,10,16-trione,
17-Ethanalyl-1,2,14-trihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl-1-methylvinyl-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-3,10,16-trione,
1,14-Dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-26,28-dimethoxy-18-[(phenylseleno)-methyl-13,22,24,30-tetramethyl-11,17,31-trioxa-4-azatetracyclo[25.3.1. 0.^{4,9}0.^{16,20}]hentriaconta-16(20),21-diene-2,3,10-trione,
Benzenesulphonic acid, 4′-methyl-[17-Allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10-trione-16-ylidene]hydrazide,
or a pharmaceutically acceptable salt of any one thereof.

8. The use of a compound of formula I, as defined in claim 1, in the manufacture of a pharmaceutical formulation for use as an immunosuppressive agent.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 88 31 1422

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X,D	EP-A-0 184 162 (FUJISAWA) * Whole document * ---	1,8	C 07 H 19/01 C 07 D 498/18 C 07 D 498/22 A 61 K 31/70 A 61 K 31/33 // (C 07 D 498/18 C 07 D 311:00 C 07 D 273:00 C 07 D 221:00) (C 07 D 498/22 C 07 D 311:00 C 07 D 307:00 C 07 D 273:00 C 07 D 221:00) (C 07 D 498/22 C 07 D 311:00 C 07 D 273:00 -/-
A	EP-A-0 227 355 (UNIVERSITY OF KANSAS) * Pages 1-2 * -----	1,8	
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
			C 07 H 19/00 C 07 D 498/00 A 61 K 31/00

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	20-03-1989	BRENNAN J.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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D : document cited in the application
L : document cited for other reasons

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European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 88 31 1422

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
			C 07 D 221:00 C 07 D 209:00) (C 07 D 498/22 C 07 D 311:00 C 07 D 303:00 C 07 D 273:00 C 07 D 221:00)
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	20-03-1989	BRENNAN J.

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X : particularly relevant if taken alone
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